PRACTICAL METHODS IN MICROSCOPY

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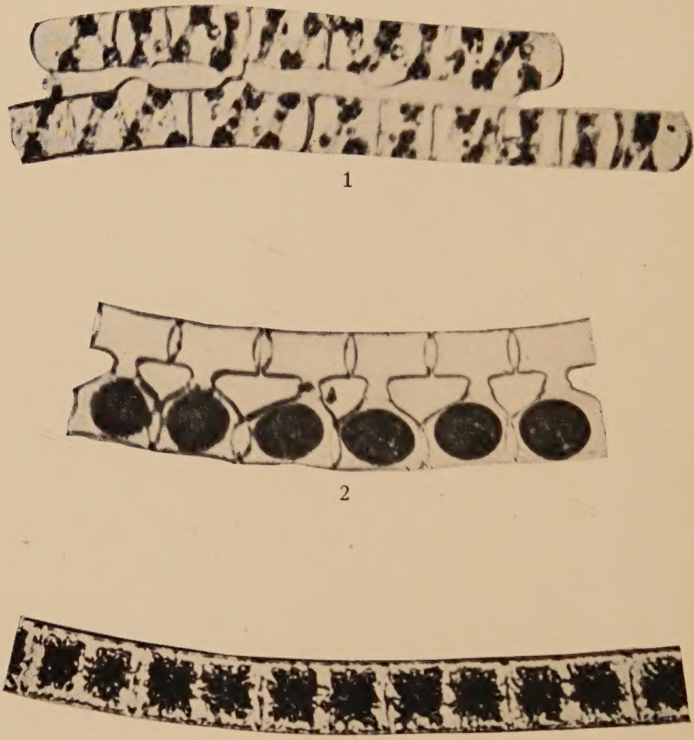

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PLATE I.

PRACTICAL METHODS

IN

MICROSCOPY

BY

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Instructor in Phillips Exeter Academy

FIFTH EDITION
REVISED AND
ENLARGED

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PREFACE.

THERE are many excellent books on the microscope, and many on special subjects requiring the use of the microscope as a working tool for investigation. In the most of these books, however, the descriptions of methods are so interwoven with other matters that the inexperienced student is confused and, in many cases, is unable to separate the essential from the non-essential. Too much is assumed to be known, or is left to be filled in by an instructor. None of these books gives to the private worker, in simple and concise language, detailed directions for the many processes that he must learn in order to make practical use of the microscope.

A large amount of apparently unproductive work must be done before the microscope can be advantageously used as a working tool. It is of the highest importance that this work be done at as early a stage as possible, and without waste of time. This book is professedly for beginners and private workers, and is designed to afford the means of acquiring this necessary training. The methods here described are practical methods which have stood the test of general use. They are the methods employed by scientific men the world over. It would not be possible, in most cases, to give credit to the originators of the processes, as the processes have been the result of slow growth, and have been so modified by different workers that they have become common property. Credit can, therefore, be given only in this general way to the books and workers that have furnished the material which has been drawn upon.

These pages are the outgrowth of the author's experience in the use of the microscope in the various branches of scientific study pursued in the secondary schools. They are the result of many hours of pleasant work, and of many hours of laborious reading in the search of what, in many cases, could not be found in the books consulted.

Only so much of the mechanical construction of the microscope and of the theory of light is given as seems absolutely essential to an intelligent understanding of the instrument; other books treat exhaustively of these subjects. The theory of polarized light has been somewhat fully considered, in part because of its important applications and great interest, and in part because this subject is so briefly and so vaguely treated in most books on the microscope. The subject is deep, and it is not easy to treat it in a popular way. An attempt has been made, while adhering to scientific accuracy, to explain in simple language the principal phenomena of polarized light so far as they have practical application in the use of the microscope.

The author wishes to express his sincere thanks to the following gentlemen who have carefully read the proof and offered many valuable suggestions: Professor Leslie A. Lee, Bowdoin College; Dr. S. G. Shanks, Albany, N.Y.; Principal Clarence E. Kelley, High School, Haverhill, Mass.; Dr. S. G. Bonney, Denver, Col.; Mr. George L. Chandler, High School, Newton, Mass.; Rev. J. D. King, Ph.D., Cottage City, Mass.; Dr. W. H. Sylvester, Natick, Mass.

Acknowledgment is also made to the Bausch and Lomb Optical Co., Rochester, N.Y.; Queen & Co., Philadelphia; and Williams, Brown & Earle, Philadelphia, for the use of electrotypes illustrating microscopes and accessory apparatus.

C. H. C.

KINGSTON, N.H., December, 1893.

PREFACE TO THE SECOND EDITION.

THIS book, as originally written, was intended for beginners working privately without the assistance of experienced instructors and without the apparatus of a well-equipped laboratory. It was, therefore, thought best not to include processes which require expensive accessory apparatus and a considerable outfit of chemicals. The complicated processes necessary for the best results with delicate tissues were also omitted as beyond the needs of the mere beginner and as likely to discourage him in his first efforts.

The favor with which the book has been received in the laboratories of several leading colleges has led to the additions which have been made in this edition to include the processes of microscopic technique that are needed in such laboratories. While the book has been extended to meet the needs of more advanced workers, it is believed that it is none the less adapted to the needs of beginners and private workers.

The author wishes to make especial acknowledgment of indebtedness for suggestions to Professor Frederick C. Newcombe, of the University of Michigan, and to Professor F. D. Kelsey, of Oberlin College.

C. H. C.

WABAN, NEWTON, Mass., May, 1896.

PREFACE TO THE FIFTH EDITION.

In the thirty years that have passed since the first edition of this book was published there has been much advance in perfecting microscopic apparatus, in the use of the microscope and photomicrography in scientific research and especially in practical application in several of our most important industries. During these years the writer has found in microscopy and photomicrography a very pleasant avocation and recreation in the intervals of an otherwise very busy life as a teacher and embodies in this edition the results of his experience. A careful study of the best modern methods has been made, and the aim has been to bring the book fully up to date.

If the way of others is made easier, the object of these pages will have been accomplished.

Thanks are extended to the Bausch and Lomb Optical Company, Spencer Lens Company and E. Leitz for electrotypes of apparatus.

C. H. C.

Exeter, N. H., June, 1924.

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Fig. 1. Spirogyra x 350, showing the beginning of Conjugation.

An enlargement on Carbon Black paper from the negative used for Plate XII. Fig. 5 (see description page xxii).

Fig. 2. Spirogyra x 350.

An enlargement on Enameled Bromide paper from a negative made at 80 diameter with the apparatus shown in Fig. 62.

Fig. 3. Zygnema x 350.

An enlargement on Lyko paper from a negative made at 100 diameters with the apparatus shown in Fig. 63.

The photomicrographs in Plates II–IX, which were published in the first edition of this book, were taken with the simple arrangement of apparatus shown in Fig. 50. The light used was an ordinary kerosene lamp; objective, a Bausch and Lomb, ¾ in.; no eye-piece; no auxiliary condenser; no sub-stage condenser; the dry plate was the Carbutt B, an ordinary plate; the average exposure was eighteen seconds; the magnification about thirty diameters; various developers were used, the tray and inspection method being employed. They illustrate what may be done in low-power work with ordinary plates, no ray filter and the simplest possible arrangement of apparatus.

PLATE II.

Fig. 1. Peduncle of Yellow Water-Lily, *Nuphar advena* 329

Part of transverse section showing stellate cells or internal hairs, fibro-vascular bundles, large intercellular spaces, and the circular sections of characteristic uncompressed cylindrical cells.

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Stained with picro-carmine, x 150. On the right are seen the cells of the epidermis; next to these in cross section the fibres of a muscle that runs in the circular direction around the body; on the left the fibres of a muscle that runs lengthwise of the body. Apparatus as in Fig. 59. Bausch and Lomb light yellow ray filter; Leitz III objective; Spencer 6 x eye-piece; Abbe condenser; Standard slow orthochromatic plate; exposure, 1 sec.; developer, elon-hydroquinone. The color of this specimen calls for a pure green ray filter to secure maximum contrast. With specimens deeply stained with this color, however, the yellow filters are quite satisfactory.

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Plate XII.

A few Algæ.

Fig. 1. *Vaucheria dichotoma;* an antheridium x 100 349

This plant is found in dark green, felt-like mats on half submerged muddy banks; also in running water attached to

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sticks and stones. Taken from a fresh specimen mounted in a drop of water. Apparatus as in Fig. 62. Leitz III objective; Spencer 6 x eye-piece; Abbe condenser; Standard slow orthochromatic plate; kerosene lamp; dark yellow gelatine ray filter; auxiliary condenser, a plano-convex lens arranged for parallel light; exposure, 1¼ min.; developer, elon-hydroquinone.

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The two parts of the figure are from different plates, both Standard slow orthochromatic. Apparatus as in Fig. 62. Leitz III objective; Spencer 6 x eye-piece; Abbe condenser; kerosene lamp; dark yellow gelatine film filter;

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This lamp can be adjusted only by great good luck so as to avoid multiple images; it is not a good light to use.

PLATE XIV.

A few Diatoms

These photomicrographs are given to show results that may be expected with ordinary achromatic objectives, uncorrected eye-pieces and Abbe condensers. The apparatus is shown in Fig. 59. Spencer 1.8 mm. oil immersion objective for Fig. 3; Leitz III objective for all others; Spencer 6 x eye-piece for all; Abbe condenser; developer for all, elon-hydroquinone.

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Fig. 2. *Coscinodiscus robustus* x 150 353
Standard slow orthochromatic plate; Bausch and Lomb light yellow ray filter; exposure, 2 secs.

Fig. 3. A small portion of Fig. 3, x 1560. . . . 353
Standard slow orthochromatic plate; Bausch and Lomb light yellow filter; exposure, 3 min.

Fig. 4. *Navicula viridis* x 150 353
Standard slow orthochromatic plate; liquid ray filter composed of 3 parts of a saturated solution of copper acetate and 1 part of a saturated solution of potassium bichromate, used in a cell 2 mm. thick; exposure, 1½ secs.

Fig. 5. *Isthmia nervosa* x 150 353
Imperial process plate; Wratten K_1 (light yellow) filter; exposure, 16 secs.

Fig. 6. *Terpsichore musica* a 150 353
Standard slow orthochromatic plate; Bausch and Lomb light yellow filter; exposure, 1½ secs.

PLATE XV.

Fig. 1. Crystals of Potassium bichromate x 125 . . 355
Apparatus as in Fig. 63; Leitz III objective; Spencer 6 x eye-piece; Abbe condenser; Seed L. Ortho plate; Wratten G + H filters (pure green light); exposure, 2 min.; developer, pyro-soda.

Fig. 2. Granite from Biddeford, Me. x 125 . . . 355
Apparatus, dry plate, ray filters, exposure and development as for Fig. 1.

PLATE XVI.

Fig. 1. Human Blood in Pernicious Anaemia x 1560 357
Apparatus as in Fig. 59. Spencer 1.8 mm. oil immersion objective; Spencer 6 x eye-piece; Abbe condenser; Standard orthonon plate; Wratten B (green) filter; exposure, 12 min.; developer, pyro-soda.

The oval and pear-shaped distortions of the red corpuscles, characteristic of the disease, are seen. At the centre is a polymorphonuclear leucocyte (white corpuscle) or phagocyte). Stained with Wright's Blood Stain.

The red blood corpuscles were very faintly stained. Blood corpuscles, if faintly stained, will be correspondingly faint in the photograph.

Fig. 2. Trypanosomes in the Blood of a Rat x 1560 357

Apparatus as in Fig. 59. Spencer 1.8 mm. oil-immersion objective; Spencer 6 x eye-piece; Abbe condenser; Wratten "M" plate; Wratten B (green) filter; exposure 36 min. (see page 213); developer, pyro-soda.

Specimen stained with Hastings's Blood Stain. The blood corpuscles were deeply stained, and on this account give good contrast with the green filter.

There are many varieties of trypanosomes infesting the blood of different animals and of man, some apparently harmless to their hosts, others causing various febrile diseases. To one variety is due the Sleeping Sickness of Tropical Africa.

Plate XVII.

Malaria Parasites in Human Blood Corpuscles.

Apparatus as in Fig. 59. Spencer 1.8 mm. oil immersion objective; Spencer 6 x eye-piece for Figs. 1, 2, 3, 4, 5, 6 and 9. Spencer 5 x compensating eye-piece for Figs. 7 and 8. Abbe condenser; Standard slow orthochromatic plate for all; developer for all, elon-hydroquinone.

Specimen stained with Wright's modification of Leishman's blood stain. The corpuscles were very faintly stained. With the apparatus arranged to utilize the full numerical aperture of the objective the light was too feeble to permit of the use of the proper ray filters to give good contrast between the blood corpuscles and the background. The Wratten B + E or G + H filters would undoubtedly have given much better contrast, if they could have been used. The various filters used were tried in the hope of securing better contrast and also in the hope of reducing the time of exposure.

PAGE

Fig. 1. Signet Ring Form of the Tertian Parasite a few hours old x 1560 359

Wratten B (green) filter; exposure, 12 min.

Fig. 2. Signet Ring Form of the Tertian Parasite slightly older than the preceding x 1560 . 359

Liquid ray filter composed of 3 parts of copper acetate and 1 part of potassium bichromate used in a cell 2 mm. thick; exposure, 6 min.

Fig. 3. Well developed Tertian Parasite showing stipling x 1560 359

Ray filter as for Fig. 2; Exposure, 6 min.

Fig. 4. Well Developed Tertian Parasite x 1560 . . 359

Ray filter as for Figs. 2 and 3; exposure, 6 min.

Fig. 5. More Advanced Tertian Parasite x 1560 . 359

Segmentation is beginning. Liquid used in filters for Figs. 2, 3 and 4 diluted with 10 volumes of water used in cell 2 mm. thick; exposure, 3 min.

Fig. 6. Fully Developed Tertian Parasite, probably about 40 hours old 359

Segmentation is well advanced, x 1560. Ray Filter as for Fig. 5; exposure, 3 min.

Fig. 7. Young Signet Ring Form of the Aestivo-autumnal Parasite x 1400 359

Wratten G (strong yellow) filter; exposure, 4 min.

Fig. 8. The Crescent Form of the Aestivo-autumnal Parasite x 1400 359

Wratten G filter; exposure, 4 min.

Fig. 9. Another Crescent x 1560 359

Wratten B (green) filter; exposure, 12 min.

PLATE XVIII.

PAGE

Fig. 1. Tubercle Bacilli in Sputum x 1225 . . . 361

Apparatus as in Fig. 65. Spencer 1.8 mm. oil-immersion objective; Spencer 6 x eye-piece; Abbe condenser; Wratten "M" plate; Wratten B (green) and E (orange) filters used together; exposure, 3 mins. (see page 218); developer, pyro-soda.

Specimen stained with carbol fuchsin, counterstained in aqueous methylene-blue and mounted in balsam.

With the apparatus as arranged the numerical aperture of the Abbe condenser was not fully utilized, and consequently the numerical aperture of the objective was so much cut down that slight diffraction bands show in the photograph around the edges of the bacilli. Iris diaphragm wide open.

Fig. 2. Bacteria from an Infusion of Cabbage Leaves x 1560 361

Stained with gentian-violet and mounted in balsam. Apparatus as in Fig. 59. Spencer 1.8 mm. oil immersion objective; Spencer 6 x eye-piece; Abbe condenser; Standard slow orthochromatic plate; Wratten B (green) filter; exposure, 12 min.; developer, elon-hydroquinone.

The apparatus as arranged allows the full use of the available numerical aperture of the Abbe condenser, and no diffraction bands show about the edges of the bacteria. Iris diaphragm wide open. The use of ray filters in pairs, which would be required with some stains or with faintly stained preparations, is not possible with this arrangement, if the 24-watt lamp is used.

PLATE XIX.

Bacteria of Decaying Vegetation x 1560

These are given to illustrate results on the Wratten "M" plate with different ray filters; also to illustrate the limitations of the 24-watt lamp for high-power work with the oil immersion objective. All the exposures are excessively long. The use of the 36 minutes exposure with the Wratten B (green) filter is quite out of the question for practical use, as

PAGE

trouble from vibrations and change of focus are increased as the length of the exposure is increased. The use of the filters in pairs is altogether impossible, and consequently correct contrast with various combinations of stains cannot be secured.

The bacteria photographed were prepared by allowing the vegetable substance to decay in water exposed to the air for three or four days. The bacteria have developed from spores deposited from the air of those that were on the vegetable matter. The smears on slides were stained in gentian-violet and mounted in balsam. It is better to make the smears on cover-glasses, as the bacteria will then lie more evenly in the same optical plane. Apparatus as in Fig. 59. Spencer 1.8 mm. oil immersion objective; Spencer 6 x eye-piece; Abbe condenser; developer for all, pyro-soda.

Fig. 1. From Oats 363

Wratten G (strong yellow) filter; exposure, 9 min.

Fig. 2. From Onion 363

Wrattan E (orange) filter; exposure, 12 min.

Fig. 3. From Hay 363

Wratten K (light yellow) filter; exposure, 4½ min.

Fig. 4. From Lettuce Leaves 363

Wratten G (strong yellow) filter; exposure, 9 min.

Fig. 5. From Potato 363

Wratten E (orange) filter; exposure, 12 min.

Fig. 6. From Pea 363

Wratten B (green) filter; exposure, 36 min.

The Wratten "M" plate is a very superior plate to use in photographing bacteria, especially with a strong yellow or orange filter, or with the green and orange used together.

To photograph well the bacteria must be sharply stained. With faintly stained specimens it is impossible to secure the proper contrast with the background.

PRACTICAL METHODS IN MICROSCOPY.

CHAPTER I.

LIGHT AND THE MICROSCOPE.

The Undulatory Theory of Light. — The Undulatory Theory of Light assumes that all space is filled with a very rare medium called the ether, which permeates every substance in the universe. The Molecular Theory of Matter assumes that every body of matter is made up of exceedingly minute particles called molecules, which are in a state of constant vibration, moving back and forth in all directions. If a body is heated, the motion of the molecules increases in rapidity as the temperature rises. When this vibratory motion has attained a certain rapidity, the body becomes luminous. This results from the fact that the motion, originating in the molecules of the body and communicated by these molecules to the particles of ether which permeate the body or lie adjacent to it, produces wave motions in the ether, which the ether transmits to the eye. It is known from experimental demonstration that, as the temperature of a body is raised, the wave motions become more rapid, and that the wave lengths in the ether are shortened. When the waves are of lengths within certain fixed limits, which have been determined by experiment, they affect the fibres of the optic nerve spread out to form the retina of the eye, and produce the sensation of light. Light is therefore defined, in a subjective sense, as a sensation produced on the retina of the eye by ether waves of certain lengths; in an objective sense, it is a form of energy causing wave movements in the ether.

It is believed that the particles of ether producing these movements vibrate in all directions transversely to the direction in which the light travels, and that the vibrations are sometimes in straight lines, sometimes in ellipses or circles, the particular kind of vibration depending upon the particular vibration of the molecules of the source of the light, or upon the influence of the molecular structure of the bodies through which the light is transmitted on the original wave movement.

The Undulatory Theory fails in one particular. If the waves are caused by the vibrations of the particles of ether, since the light travels because of the action of these particles on one another, there should be a tendency to produce not only transverse vibrations but also vibrations in the longitudinal direction. There is no evidence that there are any longitudinal vibrations. The Electro-Magnetic Theory, first proposed by Clerk Maxwell, and now generally accepted by scientists, modifies the Undulatory Theory by supposing a periodic change in the electrical and magnetic condition of the particles of the ether. In accordance with this theory there is no reason to suppose that there are longitudinal vibrations. The primary difference in the two theories relates to the nature of the cause of the vibrations. Since transverse vibrations are supposed by both theories, the explanation of the phenomena resulting from these vibrations is the same for both.

If a beam of light is allowed to enter a darkened room through a small aperture, its path through the room is made visible by the light reflected from the particles of dust in the atmosphere. The path is seen to be straight. The path of light in any homogeneous medium is straight, and its velocity uniform. If, however, the beam of light is made to pass from one medium to another of different density in any other direction than at a right angle to the surface bounding the two media, its path will be bent at the bounding surface, and its velocity diminished or increased according as the second medium is more or less dense than the first medium.

Refraction of Light. — If we place a triangular prism of any transparent substance in the path of a beam of light entering a darkened room, it is easy to see that the beam is bent at the surface of the prism both on entering and on leaving it, unless the light strikes the surface of the prism at right angles. Figure 1 shows the path of such a beam of light. The ray of light *AB* strikes the surface *EG* of a glass prism at an oblique angle. It is bent or refracted at the surface *EG* towards the base of the prism, passes in a straight line through the glass to the surface *GF*, and is refracted again towards the base of the prism.

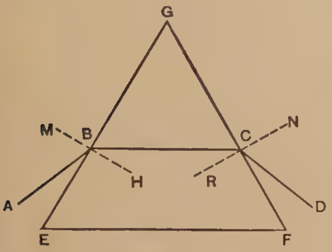


FIG. 1.

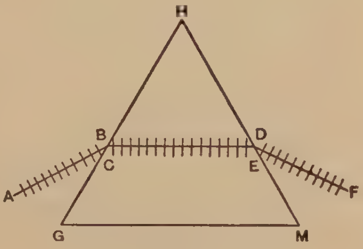


FIG. 2.

Figure 2 shows Professor Tyndall's way of making clear why the ray is bent. As a wave front approaches the surface *GH* of the prism, one end *C* of the wave reaches the denser medium while the end *B* is still in the rarer medium. In the denser medium the rate of motion is less than in the rarer medium. The end *C* moves at a slower rate than the end *B* until *B* reaches the glass. A change of direction is the necessary result. When the wave front reaches the surface *HM*, the end *D* of the wave reaches the rarer medium while the end *E* is still in the denser medium. The end *D* moves more rapidly than the end *E*, until *E* reaches the air. Another change of direction results at this surface. It is clear that if the ray falls perpendicularly on either surface, it will not be bent, as both ends of the wave strike the surface at the same instant. It is also easy to see that the more obliquely the ray strikes the surface, the greater will be the refraction. These theoretical facts are easily verified by experiment.

Returning to Fig. 1, the ray, in passing from the air into the glass, is refracted towards the line *BH* erected perpendicular to the surface bounding the two media at the point *B*, the point at which the ray enters the prism. The angle *ABM* is called the angle of incidence for the ray; the angle *HBC*, the angle of refraction. In passing from the glass into the air, the ray is refracted from the perpendicular *CN*. In general, a ray of light in passing from a rarer to a denser medium is refracted towards the perpendicular erected on the boundary surface of the two media at the point of incidence of the ray; and in passing from a denser to a rarer medium, the ray is refracted away from the perpendicular. The greater the difference in density of the two media, the greater the amount of the refraction.

Dispersion of Light. — It is also to be noted that a ray of white light passing through a prism is dispersed or divided into its primary or rainbow colors. This results from the fact that the waves of white light are compound, each wave being the result of the combination of several waves of different lengths. These different waves are differently retarded by the denser medium, the shortest waves, or those which produce the sensation of violet color, being most retarded and as a consequence most refracted; the longest, or those producing the sensation of red, being least retarded and consequently least refracted. The light, then, in coming from the prism exhibits all the colors of the rainbow.

The dispersive powers of different substances are very different. Flint glass has nearly twice the dispersive power of crown glass. This suggested to Dollond, a London optician, a means of effecting refraction without dispersion. He combined a crown glass prism *ABC* with a flint glass prism *CDB*, Fig. 3, with the edges turned in opposite directions. By a careful adjustment of the size of the angle *CBD* to the angle *ACB*, the rays which are separated in the prism *ABC* are caused to combine in the prism *BCD*. In this way refracted light which is nearly but not quite achromatic is obtained. This principle has an important application in the construction of achromatic objectives.

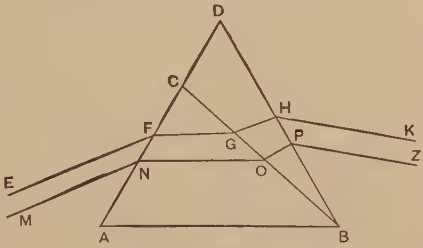


FIG. 3.

Index of Refraction. — Let *AB*, Fig. 4, represent the boundary surface between two media, *e.g.* glass below, air above. *CO* is a ray of light entering the glass at *O*. It is refracted towards the perpendicular *OR* and passes along the line *OD*. With *O* as a centre and with any radius describe a circle, as *AEBF*. From the point *M* where the incident ray cuts the circumference draw *MH* perpendicular to *EF*. The angle *COE* is the angle of incidence, and *MH* is the trigonometrical sine of the angle of incidence for the ray *CO*. From the point *P* where the refracted ray cuts the circumference, draw *PR* perpendicular to *EF*. *POF* is the angle of refraction, and *PR* the sine of the angle of refraction for the ray *CO*.

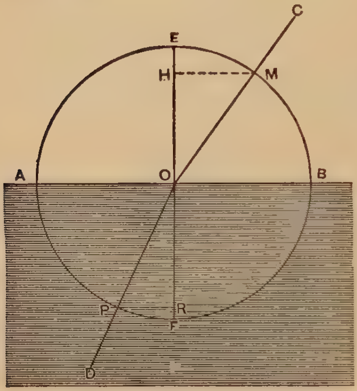


FIG. 4.

Notwithstanding that it is true that the refraction of the ray increases as the angle of incidence increases, it has been found by experimental demonstration that, as long as the two media remain the same, the ratio of the sine of the angle of incidence to the sine of the angle of refraction is constant. This ratio $\frac{MH}{PR}$ is called the Index of Refraction. It is usually expressed decimally. If the ray is passing from a rarer to a denser medium, the index of refraction is greater than unity; if the ray is passing from a denser to a rarer medium, the index of refraction is less than unity.

The index of refraction for a ray of light passing from a vacuum into any medium is called the absolute index of refraction for that medium. The absolute indices of various substances have been experimentally determined. The following table, compiled from several authentic sources, gives the absolute indices of refraction for some of the media that are of importance to the microscopist: —

Absolute Indices of Refraction.

Air	1.000294	Plate glass (glass slips and cover-glasses)	1.53
Pure water	1.333	Homogeneous immersion fluid	1.51
Canada balsam	1.54	Iceland spar, ordinary ray	1.654
Oil of cloves	1.53	Iceland spar, extraordinary ray	1.654–1.483
Oil of turpentine	1.478		
Oil of cedar	1.51		
Crown glass	1.51		
Flint glass	1.62		

The relative index of refraction for a ray of light passing from one medium to another is found by dividing the absolute index of the medium into which it is passing by the absolute index of the medium from which the ray is coming. If the ray is passing from a rarer to a denser medium, this quotient is greater than unity, and the ray is refracted toward a line erected at the point of incidence perpendicular to the surface bounding the two media. If the ray is passing from a denser to a rarer medium, the quotient is less than unity, and the ray is refracted away from the perpendicular. As the index of refraction shows at a glance which way the ray is bent, and whether it is bent much or little, it is a very important datum. An understanding of the index of refraction is of great practical importance in studying immersion objectives and the effects of the cover-glass.

Total Reflection. — Intimately connected with refraction is total reflection, which, because of its practical bearing in the construction of the polarizing apparatus used on the microscope, requires attention here. Place the eye on a level with a gas or lamp flame and raise a glass of water steadily above this level between the eye and the flame. When the water is held in a particular position, the flame is seen reflected as from a mirror from the under part of the upper surface of the water. Fig. 5 shows the reason. Let *AB* be the surface of the water. The ray of light *EO* in passing from the water into air is refracted away from the perpendicular *CO* along the line *OF*. The ray *GO* is refracted along *OH*, the angles of refraction increasing more rapidly than the angles of incidence. If we continue to take the successive rays, a ray will be found, as *B′O*, which strikes the surface *AB* at such an angle that the angle of refraction *COB* is a right angle. The refracted ray just grazes the surface of the water, passing along the line *OB*. All rays between *B′O* and *AO* cannot pass out of the water, but are totally reflected from the surface. The angle of incidence *B′OD* is called the critical or limiting angle. When the light strikes the surface of a transparent medium at an angle greater than the critical angle, this surface is a perfect mirror. The critical angle for water is about 48° 30′. It is different for different substances. For the diamond it is 23° 41′. The brilliancy of the diamond and other gems is due to repeated total reflections of the light within the gem.

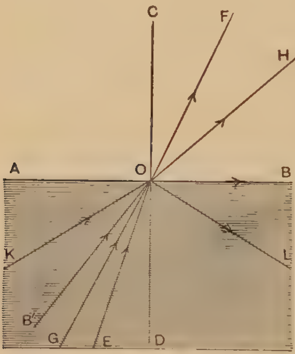


FIG. 5.

The Double-Convex Lens.—Fig. 6 represents two prisms placed base to base and the paths of rays of light from the point *F*. It is seen that the rays which diverge from the point *F* converge, after passing through the prism, to the point *A*. This arrangement of prisms represents very well the principle of the double-convex lens.

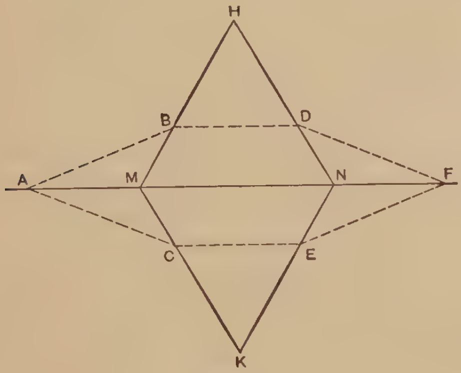


FIG. 6.

The surfaces of a double-convex lens may be considered as made up of an infinite number of small plane surfaces. Each ray of light coming from a given point, except the one passing along the optic axis *BA*, Fig. 7, is refracted towards the optic axis both on entering and on leaving the lens. The result is that rays coming from the point *B* are refracted to the point *A*.

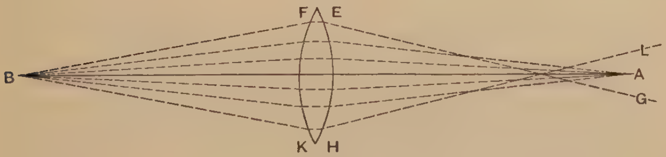


FIG. 7.

Spherical Aberration. — The surfaces of convex lenses are usually portions of spheres. As the incident rays approach the edge of the lens, the angles of incidence increase very rapidly, and the refraction of the rays is, of course, correspondingly increased. The result is that those rays which pass through the lens near its edge, as *BF* and *BK*, Fig. 7, are brought to a focus sooner than the rays which pass through the central portion of the lens. There results a blurring of the image. This defect is in part remedied by combining flint and crown glass so shaped that spherical aberration and dispersion are both corrected. Spherical aberration may also be partially connected by a diaphragm to cut off these objectionable rays. Diaphragms are used in all the optical parts of the microscope, as the eye-piece, objective, condenser.

Dispersion in Lenses. — Another defect in convex lenses of homogeneous material is dispersion of the light, whereby color effects due to the separation of white light into its primary colors are produced. From the shape of the lens this separation is not so great as in the case of the prism, yet there is enough to give circles of prismatic colors around the edge of the field of view. This defect is remedied by combining, on the principle of Dollond, convex and concave lenses of crown and flint glass so adjusted that the dispersion of the one is corrected by the opposite dispersion of the other, in a manner similar to that already explained under prisms. The correction can, however, be perfectly made for only two colors at a time, so that lenses entirely free from dispersion cannot be made. The skill of the opticians has produced lenses which would seem to be as free from this defect as human ingenuity can make them. Fig. 8 shows such a compound lens. Ordinarily the convex lens is of crown glass and the plano-concave lens of flint glass, but for some purposes the order is reversed. These lenses are called achromatic, and are used in the construction of all optical instruments of high grade. Apochromatic objectives are a modern triumph of the opticians in which the correction is practically made for three colors.

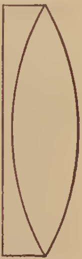


FIG. 8.

The Simple Microscope. — Every double-convex lens is a simple microscope. The manner in which it acts is illustrated in Fig. 9. A ray of light coming from the point *A* on the arrow *AB*, placed just within the principal focus of the lens, is refracted on entering the lens and again on leaving it, passing in the line *HK* to the eye. The eye looking along the line *KHA'* seems to see the point *A* at *A'*, the position being determined by the intersection of the line *KA'* and the secondary axis *PA'*; similarly the point *B* appears at *B'*. In like manner we could draw lines to show that all the rays coming from all the points of *AB* would be so refracted that they would appear to come from points on *A'B'*. We thus see an enlarged image of the arrow *AB* in the position *A'B'*. It is to be noted that the image is not inverted.

A simple microscope consists, then, of one or more convex lenses mounted in such a way as to be convenient to use for the purpose for which the microscope was designed. Any lenses of the convex series — double-convex, plano-

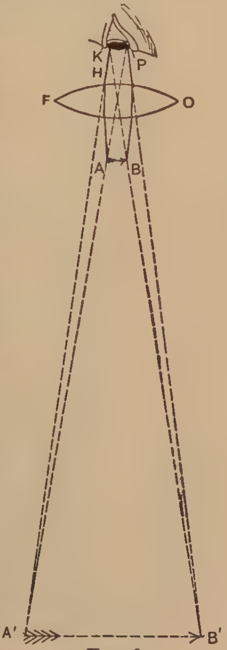


FIG. 9.

convex, or concavo-convex — may be so employed. The manner of action is precisely the same in all, but is less in amount in the plano-convex and concavo-convex lenses than in the double-convex. Figs. 10, 11, and 12 show different manners of mounting simple microscopes.

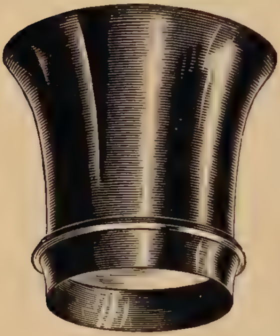


FIG. 10.

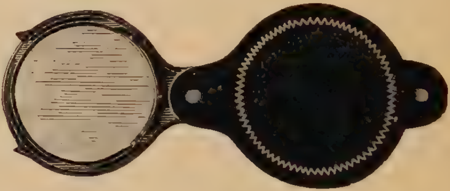


FIG. 11.

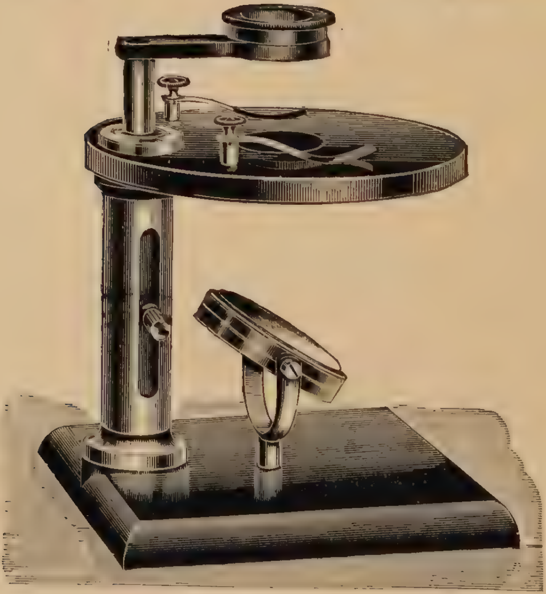


FIG. 12.

The Compound Microscope. — The simplest form of compound microscope consists of two convex lenses: one small and placed near the object; the other larger and placed in such a position that the image formed by the small lens is in its focus. The small lens is called an objective from the fact that it is placed near the object; the larger lens, an eye-piece or ocular, from the fact that the eye is placed near it to make observation. Fig. 13 shows how the objective forms a real, inverted image of the object *AB* at *A'B'*. If a screen of oiled paper or delicately ground glass is placed here, the inverted image

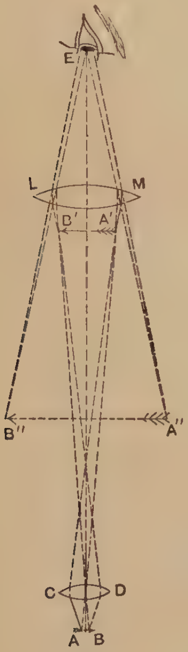


FIG. 13.

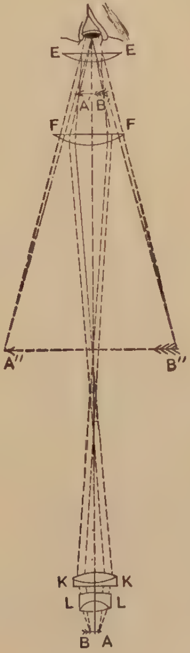


FIG. 14.

of the object will be seen upon it. The eye-piece acts as a simple microscope to magnify this image.

In practice, an objective consists of two, three, or four achromatic convex lenses placed near together. The lens nearest the object is called the front lens or front combination; the one farthest from the object is called the back combination. These different lenses act together to magnify the object. Two or more lenses of slight curvature are used rather than one of considerable curvature, as it is found that spherical aberration and dispersion are thus reduced to a minimum.

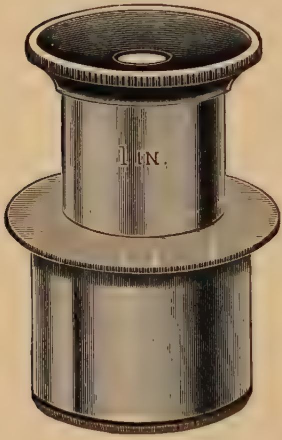


Fig. 15.

The eye-piece sometimes consists of a single convex lens. It is then known as a positive eye-piece and is really a simple microscope, as *LM* in Fig. 13. More commonly a Huyghenian eye-piece is used. This consists of two convex lenses. The one nearest the eye is called the eye-lens; the other, the field-lens. The field-lens acts precisely as one of the lenses of an objective, and is regarded by many as constituting a part of the objective. The rays of light from the object, after passing through the objective, pass through the field-lens of the eye-piece before coming to a focus. Refracted by the field-lens they form the inverted image of the object between the field-lens and the eye-lens, as $A'B'$, Fig. 14. If the eye-lens is removed and a piece of oiled paper lowered into the eye-piece, an image of the object will be seen on it when it is carefully held transversely in the tube at the proper distance from the field-lens. The diaphragm of the eye-piece is in the plane where the image is formed. When an eye-piece micrometer is used, it is placed in this position. The eye-lens acts as a simple microscope and magnifies this image. It is thus seen that with the compound microscope the image appears inverted whether the positive eye-piece or the negative Huyghenian eye-piece is used.

The compound microscope is then an application of convex lenses. The stand with all its various parts is only a convenient means to render the use of the lenses easy and bring out their full power. In Fig. 16, *A* is the objective, *B* the eye-piece, *C* the tube or body, *D* the draw-tube, *E* the nose-piece which adapts the objective to the body, *F* the stage, *G* the sub-stage which receives various accessories, *H* the mirror, *K* the mirror-bar, *J* the base, *L* the flexible pillar, *O* the arm, *M* the coarse adjustment by rack and pinion, *N* the fine adjustment. On the stage is seen a microscope slide, three inches by one in dimensions, held in position by spring clips.

Selection of a Microscope. — Much has been written in regard to the selection of a microscope, but to very little purpose. It is impossible for one person to give another very definite practical advice on such a matter. Tastes, purses, and the object for which the instrument is to be used differ. A few facts should, however, be borne in mind. Very cheap microscopes are useless for practical purposes. It is poor economy for a beginner to suppose that a cheap stand is good enough while learning to use the microscope. It is good economy, although the first outlay will be somewhat greater, to purchase a stand of thorough workmanship, of sufficient steadiness to bear any objective, and of such construction as to receive any of the sub-stage accessories. Such a stand admits of the highest character of work and of unlimited development in microscopical skill on the part of the possessor. It must, of course, be used with especial care by the beginner, but the exercise of this care is the very best means to educate one to a careful and painstaking system of working, the secret of success in microscopical manipulation. The prospective purchaser will, of course, consult his microscopical friends and learn what he can about their instruments and receive the benefit of their experience. He will also obtain the catalogues of several of the most reputable makers of microscopes, compare the instruments and prices, and will purchase only of makers of established reputation, who place their names on their apparatus as a guarantee that it is what it is represented to be.

A stand of substantial construction similar to those shown in Figs. 36 and 50, with two eye-pieces, 6 x and 10 x, and two objectives, a 16 millimeter and a 3 or 4 millimeter, is ample equipment for elementary work. As knowledge of the instrument and experience in its use are acquired other parts may be added. An error that is commonly made in first purchases is to obtain a multiplicity of accessories that are afterwards found to be useless. It is much the wiser course to purchase accessories as they become actual necessities.

Care of the Microscope. — The microscope should be kept scrupulously clean in all its parts. The metal portions may be wiped along the grain of the finish with a piece of old, soft linen. The draw-tube should be thus wiped occasionally. No alcohol or other fluid which dissolves resinous substances should be allowed to come in contact with the metal, or the lacquer finish will be dissolved.

Dust may be removed from the lenses with a piece of

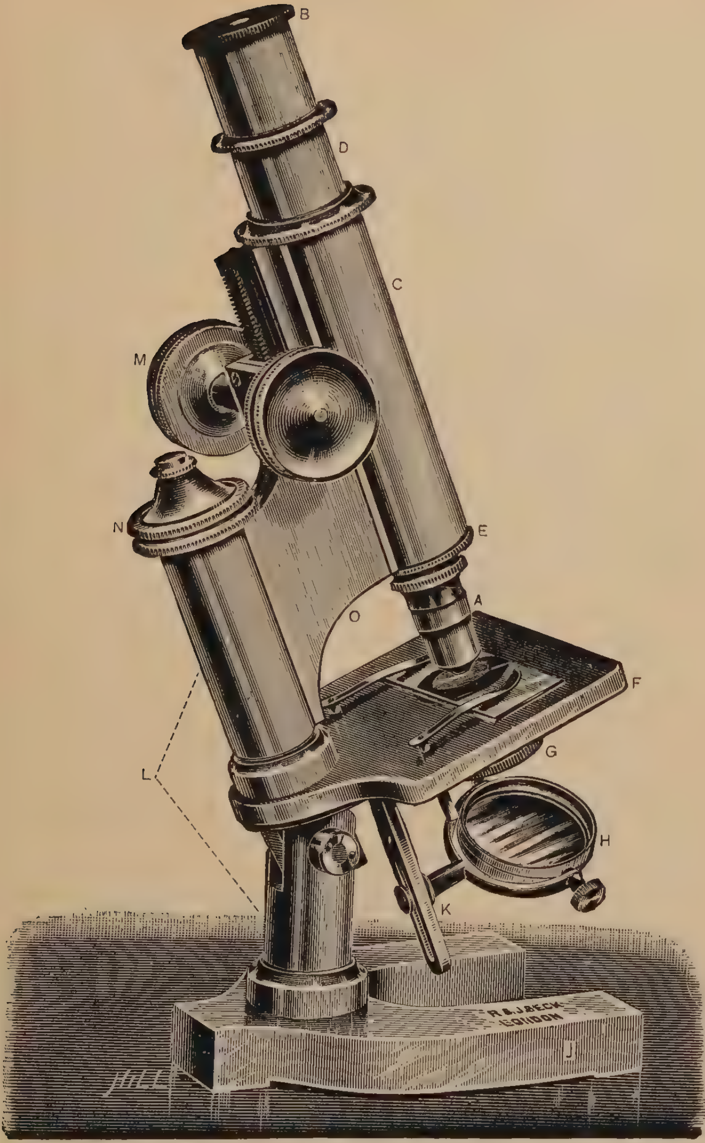


FIG. 16.

old, soft linen or a camel's-hair brush. One or two folds of the linen over the end of a stick of pine wood, previously chewed until it is quite soft, serves well to reach the smaller lenses. Professor Gage of Cornell University recommends very highly for cleaning lenses the bibulous Japanese filter paper much used by dentists. Do not touch the lenses with the bare fingers. After wiping, breathe upon the lens. If it is optically clean, the moisture will disappear almost instantaneously. If it is not clean, the moisture will persist.

To remove the objective from the stand, lightly grasp the lower part, without touching the glass itself, with the thumb and forefinger of one hand to guard against dropping the objective. Place the thumb and forefinger of the other hand on the milled rim at the upper part of the objective and unscrew it. In replacing the objective on the stand, use the same precautions. The lenses of the objective should not be separated unless one has had considerable experience with optical apparatus, and in the case of the higher power objectives it is perhaps best to send them to the makers of the instrument if they need to be taken apart for any reason. It will seldom occur that this is necessary.

In the course of use the front lens of the objective is liable accidentally to come in contact with the reagent used in the study of the specimen. It should be wiped immediately. If it has touched Canada balsam or other resinous substances, it may be wiped with a piece of linen moistened with alcohol, taking great care that the alcohol does not come in contact with the brass mounting of the objective. After using the alcohol, wipe immediately with dry, soft linen.

Specks in the field of view are most commonly due to dust on the field-lens of the eye-piece. Dust is liable to collect on both the field-lens and the eye-lens. Either lens may be unscrewed from its mounting and wiped, with the precautions already mentioned, which apply to the cleaning of any of the optical parts of the instrument.

To determine when the dust has been entirely removed, place the eye-piece in the tube, focus sharply on a well-lighted field, and then slowly revolve the eye-piece. If there are any particles of dust on either of its lenses, they will revolve, while dust specks on the slide, mirror, or objective remain stationary. To ascertain if the dust is on the slide or mirror, move first one, then the other. The dust will move with the slide or mirror, if there is any on either. If, after these manipulations of the eye-piece, slide, and mirror, there are still dark patches which have not moved, there is dirt on the objective.

The Glass Slip. — Objects to be examined under the microscope are placed upon glass slips three inches long and one inch wide. These should be purchased ready for use. Only those with ground edges should be used, and it is economy in satisfaction, if not in money, to buy a good quality. Recent improvements in the processes of manufacture, and the increased demand arising from the great advance in the popular use of the microscope in the past few years, have caused large reductions in the prices of the slips, so that the purchase of the best is now no great burden. Slips with unground edges are very liable to leave serious scratches on the stage of the microscope. Slides made from such slips are unsightly and lessen the ambition to produce perfect work. It is a waste of time, which can better be given to something else, to attempt to grind one's own slips.

The Cover-Glass. — If the object is to be examined in any fluid medium, it *must* be covered with a piece of thin glass, known as the cover-glass. This is used in part to protect the objective, in part to flatten the object, but more particularly to prevent the refraction of the light which would occur at the curved surface of an uncovered drop of fluid. This refraction of the light would cause a very distorted image of the object to be formed. The curved surface of the drop would act precisely as the curved surface of a lens. The cover-glass itself exerts a very great influence on the light, but this influence is far less objectionable than that of a distorted drop of water.

The thickness of the cover-glass is also a matter of importance. A study of Fig. 17 will make plain how the thickness of the cover-glass affects the clearness of the image. The figure is drawn for a dry mount. Let *A* represent a point on an object, and *EF* the cover-glass. A ray of light, *AB*, from the object, on striking the lower side of the cover-glass, is passing from a medium, air, whose refractive index is 1.000294, into another medium, plate glass, whose refractive index is 1.53. The relative index of refraction is 1.52. The ray is therefore refracted towards the perpendicular *MN* erected on the lower surface of the cover-glass at the point where the ray strikes. On passing from the cover-glass into air, the ray is passing from a medium whose refractive index is 1.53, into a medium whose refractive index is 1.000294. The relative index of refraction in this case is .65. The ray is

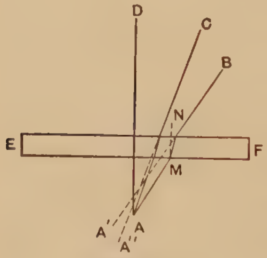


Fig. 17.

now refracted away from the perpendicular, and as the conditions in the second refraction are the exact reverse of the conditions in the first refraction, its course after leaving the upper surface of the cover-glass is parallel with its course before entering the cover-glass. The ray has suffered displacement. $A'B$ shows the direction from which it appears to come, and the point where the line $A'B$ crosses the optic axis of the microscope, DA, shows the position in which A appears to the observer in so far as this particular ray helps to form its image. It is apparent from an inspection of the figure that the greater the thickness of the cover-glass, the greater the displacement of the ray. It is also to be remembered that this displacement is magnified when observed through the microscope. The line $A''C$ shows the apparent direction of the ray AC. Should we, in like manner, construct lines for other rays coming from the point A and trace the directions back, we should see that all are displaced in varying degrees. The result is, of course, the blurring of the image.

This defect of the cover-glass can to a considerable extent be remedied in the construction of the objective. Except in the case of collar adjustment objectives, which will be considered later, it must be done for a particular thickness of cover-glass. It is accomplished by giving the lenses such shapes, and placing them at such distances from one another, that the rays which have been displaced are refracted back, so that they appear to come from their real source, the point A in Fig. 17. Microscope makers correct their non-adjustable objectives for the particular thickness of cover-glass and for the particular length of tube which they have severally adopted. It is unfortunate that the makers have not agreed upon the same thickness of cover and the same tube length. The tube length adopted by opticians of high reputation varies from 125 to 254 millimetres. The thickness of covers adopted varies from one-tenth to twenty-five hundredths of a millimetre. If an objective corrected for a cover one-tenth of a millimetre thick be used on one twenty-five hundredths of a millimetre thick, the image will be blurred, and *vice versa.* If it is necessary to use cover-glasses that are too thick or too thin, the defect can to a considerable extent be remedied by using a greater length of the draw-tube when the cover is thinner than the standard thickness for which the objective was constructed, and by shortening the draw-tube, if the stand is constructed so as to allow it to be done, when the cover is too thick. In examining permanent mounts, when the thickness of the cover-glass is not known and cannot be measured, the proper length of the draw-tube should be found by trial. Focus sharply with the tube of the length for which the objective was constructed. Then lengthen or shorten it until the clearest image possible is obtained. Such intelligent use of the draw-tube aids greatly in securing a clear image and in bringing out the highest capabilities of the objective. The foregoing considerations show that it is very important to know the thickness of the cover-glass.

Cover-Glass Gauge. — Several devices have been contrived for measuring the thickness of the cover-glass. A very commendable form has recently been placed upon the market at a reasonable price by the Bausch and Lomb Optical Company. It is shown in Fig. 18. The cover-glass is placed in the slot provided for it, and the micrometer screw turned until the end touches the cover-glass. The thickness in fractions of a millimetre or of an inch is then read directly from the drum. The drum also shows directly the correct tube length to use for any thickness of cover.

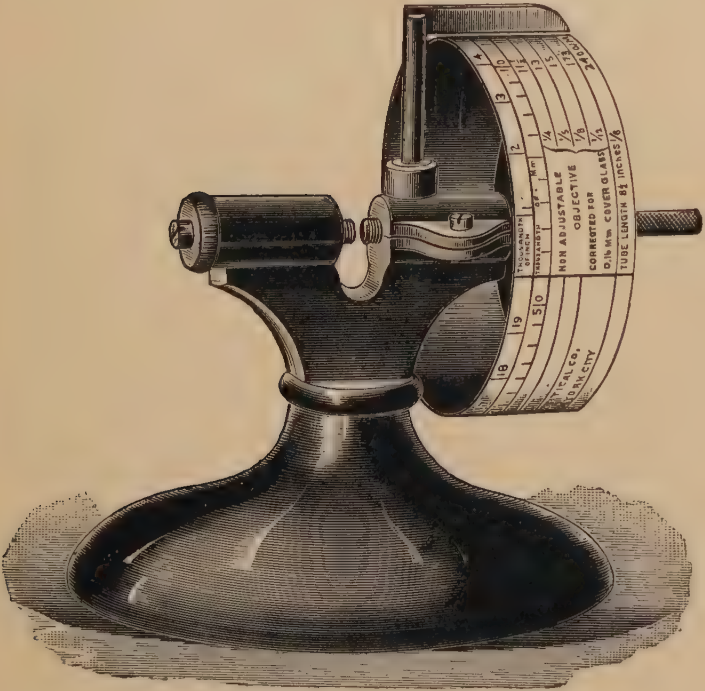


FIG. 18.

CHAPTER II.

SOME ACCESSORIES FOR ADVANCED WORK.

Immersion Objectives. — For the more advanced histological, and especially for bacteriological work, high power immersion objectives are required. These may be divided into two classes, those with which water is used as the immersion fluid, and those with which an oil is used, usually cedar oil, or some fluid especially prepared by the opticians, and having a refractive index about the same as the refractive index of the front lens of the objective. This fluid is called homogeneous immersion fluid, as it is homogeneous as respects its refracting and dispersing power with the glass of the front lens. The objective is called a homogeneous immersion objective. Objectives used with water are called water immersion objectives.

In using an immersion objective, a small drop of the fluid is placed on the front of the front lens or on the cover-glass, and the objective carefully lowered until the fluid forms a connection between the objective and the cover-glass. The objective is then focussed by means of the fine adjustment, and, if the objective is adjustable, by the collar adjustment. The principle upon which an immersion lens works and its superiority over a dry objective will be apparent from a study of Fig. 19.

A ray of light starting from the point *A* of the object on the slide, passes from the balsam to the upper surface of the cover-glass with only a slight change of direction at the lower surface of the cover-glass; for, as will be seen by inspecting the table of Indices of Refraction given in Chapter I., it is passing from a medium, Canada balsam, whose refractive index is 1.54, into another medium, the cover-glass (plate glass), whose index of refraction is 1.53, or from a denser to a slightly rarer medium. The relative index of refraction is .993, which shows that the ray is slightly bent away from the perpendicular.

If now a dry objective is used, the ray, in passing from the upper surface of the cover-glass into the air, is passing from a medium whose index of refraction is 1.53 into a medium whose index of refraction is 1.000294. The relative index of refraction is .653, which shows that the ray is much bent away from the line *CH*, erected at the point where the ray strikes the upper surface of the cover-glass perpendicular to that surface. This perpendicular is, of course, parallel to the optic axis of the microscope. The ray takes some such direction as *CP* and fails to reach and enter the front combination of the objective.

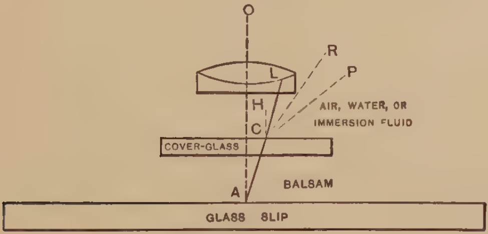


FIG. 19.

If, instead of the dry objective, a water immersion objective is used, the ray, in passing from the cover-glass into water, is passing from a medium whose refractive index is 1.53 into a medium whose refractive index is 1.333. The relative index of refraction in this case is .871.

This shows that the ray is still refracted away from the perpendicular to a considerable extent, but by no means to so great an extent as when the dry objective was used. The ray takes some such path as *CR*, and also fails to reach and enter the front lens of the objective; but it is evident that many more rays will enter a water immersion objective than can enter a dry objective of the same angular aperture and working distance.

If we use a homogeneous immersion objective, the ray, in passing from the cover-glass into the homogeneous immersion fluid, is passing from a medium whose refractive index is 1.53 into a medium whose refractive index is 1.51. The relative index of refraction is .987. The ray is but slightly refracted away from the perpendicular. It takes some such path as *CL*, reaches and enters the front lens of the objective without refraction, as the refractive index of the front lens (crown glass) is the same as the refractive index of the homogeneous immersion fluid. It is evident that the homogeneous immersion objective receives a much larger number of rays from the point *A* than does a water immersion objective of the same angular aperture and working distance.

The importance of causing the largest possible number of rays from the object to pass through the objective is manifest, when we consider that the perfection of the image depends on the number of rays that help to form it. The gain in light is also of great consequence, for, with high-power objectives having three and four combination-lenses, the absorption of light is considerable.

Adjustable Objectives. — To remedy the evil effects of changes in the thickness of the cover-glass, and to compensate for variations in the refractive index of different cover-glasses, and for changes in the tube length, which result when different eye-pieces are used, high-power objectives, both dry and immersion, are made with means of adjusting the distance between the front and back combinations of the objective. Theoretically, homogeneous immersion objectives do not require to be made adjustable, and many of them are not so made; but because the conditions for which they are made can seldom be perfectly met, because of the variation in the refractive indices of different cover-glasses, and because the immersion fluid and other factors which influence the working of the objective are liable to vary, the highest grade homogeneous immersion objectives are made adjustable: water immersion objectives are always made adjustable.

An objective is made adjustable by mounting either the back or the front combination so as to be moved up or down by means of a milled collar. This collar is graduated arbitrarily, and when the adjustment is at the medium point, the objective is corrected for the standard thickness of cover-glass, and the standard length of tube used for all objectives by the maker. When the adjustment is at the lowest point, the front and back combinations are separated and adjusted for the thinnest cover-glasses. When the adjustment is at the highest point, the lenses are brought nearest together and adjusted for thick covers. In some objectives the front combination is movable; in others, the back combination. The latter arrangement is the one now in common use, as the objective is much more easily handled.

The manipulation of adjustable objectives calls for the highest skill of the microscopist. Only practice gives proficiency in handling them. General directions can be given, but much must be left to the patient perseverance of the worker. Some useful hints on their manipulation are given in Chapter IV.

Angular and Numerical Aperture. — Since the perfection of the image depends upon the number of rays which help to form it, the efficiency of an objective depends upon the number of rays it can transmit. Dry objectives were long compared with one another by comparing the angles formed by the most divergent rays which can pass through the objective from a point on the object situated on the optic axis of the microscope. These angles are called angles of aperture. In a similar way, water immersion objectives were compared with one another, and also homogeneous immersion objectives were compared among themselves. There was even confusion, and objectives of one kind were compared with those of another kind by the angular apertures. Even when objectives of the same kind are compared, only rude ideas of the relative efficiencies are obtained. The efficiencies are really to one another as the sines of one-half the angles of aperture.

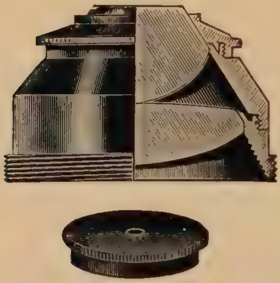
If, however, we wish to compare dry, water immersion, and homogeneous immersion objectives with one another, we must find some means of comparing the number of rays which can pass through these different kinds of objectives. Professor Abbe of Jena first demonstrated the fact that the relative efficiencies of objectives could be expressed by the numbers obtained by multiplying the sine of one-half the angle of aperture by the index of refraction of the immersion fluid employed. The result is called the numerical aperture. It is calculated by the formula $\text{N.A.} = n \sin u$, where N.A. stands for *numerical aperture*, n for the *index of refraction* of the immersion fluid employed, if the objective is an immersion objective, or of air, if the objective is used dry, and sin u, for the *sine of one-half of the angle of aperture* of the objective.

If, for example, we wish to compare three objectives each $\frac{1}{8}$-in. focus, the first, dry, angular aperture 135°; the second, water immersion, angular aperture 170°; the third, homogeneous immersion, angular aperture 138°; they do not compare as the angles 135°, 170°, and 138°, but obtaining the sines of one-half of each of these angles from a table of natural sines, and making the substitutions in the above formula, we have for the dry objective, N. A. $= 1.000294 \times .9239 = .924$; for the water immersion objective, N. A. $= 1.33 \times .9962 = 1.32$; for the homogeneous immersion objective, N. A. $= 1.51 \times .9336 = 1.41$. The efficiency of these three objectives is then as .924, 1.32, and 1.41.

By a similar application of the formula, it may be seen that objectives of 82° homogeneous immersion, 96° water immersion, and 180° dry, are practically of the same efficiency in transmitting the light from the object.

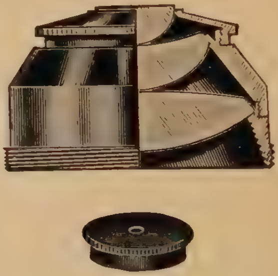
Sub-stage Condenser. — On account of the great absorption of light by high-power objectives, a sub-stage condenser must be used in the more advanced studies with the microscope. The condenser is a lens or system of lenses which converge the light upon the object. A medium-power objective makes a very good condenser when the sub-stage is so arranged that the objective can be attached with its front lens towards the object, and moved up and down until the object on the slide is in its correct focus; for, like any other lens, the sub-stage condenser, whatever its construction, must be focussed, and upon the accuracy with which this is done depends its usefulness.

For fine work, however, the condenser must be of special construction. Figs. 20 and 21 show the construction of different forms of the Abbe condenser as made by the Bausch and Lomb Optical Company. The working distance of these condensers is very short, so they must be placed close up to the slide; and as the thickness of the glass slip is variable, the condenser must be specially focussed for each new slide used. The better classes of sub-stages are provided with rack and pinion to accomplish this. Centring-screws are also provided in the sub-stage to centre the condenser correctly. In centring, a pin-hole cap, provided for the purpose, is placed over the front lens, and the centring-screws adjusted until the opening is in the centre of the field. In using the condenser, it is very important to see that it is correctly centred and correctly focussed.



Optical part of Condenser 1.20 N. A.

FIG. 20.



Optical part of Condenser 1.42 N. A.

FIG. 21.

These condensers may be converted into immersion condensers by placing a drop of water or of immersion fluid on the front lens, and making contact with the lower side of the slide.

The Camera Lucida. — The camera lucida is an attachment to the eye-piece of the microscope, whereby the image may be seen projected upon the surface of a sheet of paper. One of the simplest forms is shown in Fig. 22. To use this form, the microscope is inclined. The drawing surface must be placed parallel with the tube. The image of the object is then seen without distortion when the eye is placed so that the axial ray from it to the drawing surface is perpendicular to the latter. The principal difficulties to be overcome arise from the indistinctness of the pencil point, and from the fact that the image is inverted.

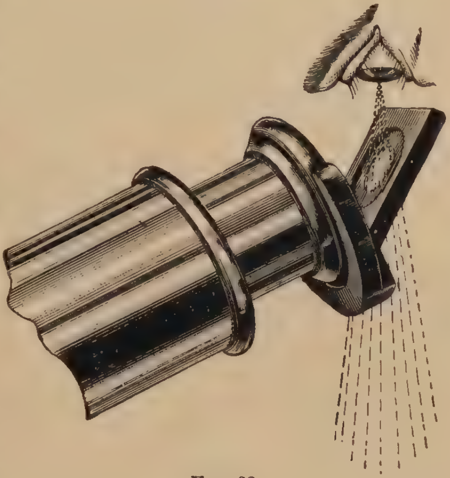


Fig. 22.

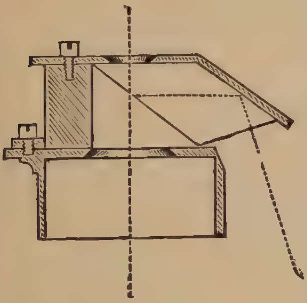


Fig. 23.

Fig. 23 shows the principle upon which the Abbe camera lucida is constructed, and Fig. 24 shows an improved form. These are the best forms of the camera lucida made. They are designed primarily to be used with the microscope in the upright position, but may also be used with the instrument inclined. To render the pencil point more distinct, a prism made up of a wedge of smoked glass and a counterbalancing wedge of clear glass is provided to be mounted in spring clips. By means of this prism a varying thickness of the smoked glass may be placed in the path of the rays which form the image, until the pencil point is distinctly visible.

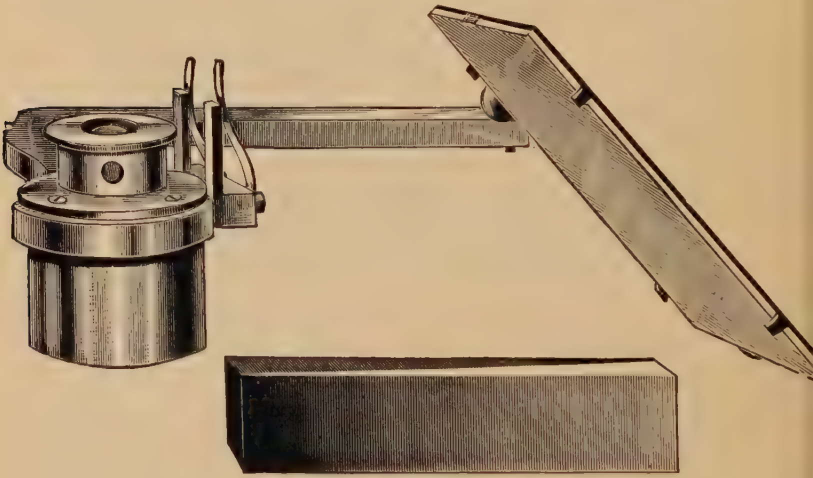


FIG. 24.

The drawing surface should be shaded from all extraneous light.

The educational value of drawing the objects which are being studied cannot be too highly estimated. In no other way can the structure be so easily and clearly fixed in the mind. Points of structure are thus frequently called to the attention which otherwise would be entirely overlooked. The process is slow and tedious, but measured by the knowledge gained the results are all valuable.

Micrometers and Micrometry. — There are two simple ways of measuring the size of an object under the microscope. The first is by means of a stage micrometer. A stage micrometer is simply a scale ruled on glass by a dividing engine. The divisions are usually $\frac{1}{1000}$, $\frac{1}{100}$, and $\frac{1}{10}$ of an inch, or, in the metric system, $\frac{1}{100}$ and $\frac{1}{10}$ of a millimetre. The stage micrometer is placed on the stage, and the object placed above it. The size is read directly. Theoretically this is the simplest way to measure an object, but practically there are almost insurmountable difficulties in the way of accurate work.

The second way of measuring is by means of an eye-piece micrometer. By this method the object is not directly measured, but the image of it formed in the eye-piece. The eye-piece micrometer is also a scale ruled on glass. The rulings are generally made $\frac{1}{10}$ of a millimetre from one another. The slip of glass is inserted in the eye-piece just above the diaphragm, and in such a position that the scale is exactly in the plane in which the image of the object is formed. As it is the image of the object and not the object itself that is measured, it becomes necessary to know how much the image has been magnified. This differs with objectives of different focal lengths, and also to some extent even with objectives marked as of the same focal length. It is necessary, then, to determine this magnification for every objective used. This is done by placing an accurate stage micrometer on the stage, and using it as an object. Observe how many of the divisions of the eye-piece micrometer correspond to one division of the stage micrometer. Naturally the way to do this is to observe some number of entire divisions of the stage micrometer that correspond to some number of entire divisions of the eye-piece micrometer, and calculate the value of a single division of the eye-piece micrometer from these data. The figures obtained are the valuation of each division of the eye-piece micrometer scale in terms of the divisions on the stage micrometer. These figures must be determined for each objective used.

With the tube at standard length it may happen that no two lines of the eye-piece micrometer correspond *exactly* with two lines of the stage micrometer. If so, it is *very important* to adjust the tube length by means of the draw tube until exact coincidence is established before making the calculations. In using the valuations thus determined, the measurements of the object must be made with the tube length used when the valuations were made.

Suppose 1.1 mm. of the stage micrometer are exactly covered by 73 spaces of the eye-piece scale. Then 1.1 mm. ÷ 73 = .015 mm., *i.e.*, one space of the eye-piece scale counts as .015 mm. The stage micrometer may now be put aside, and objects measured with the eye-piece micrometer alone. Simply multiply .015 mm. by the number of spaces of the eye-piece scale that cover the image of the object. For example, the image of the object is covered by eight spaces of the eye-piece scale. Then, .015 mm. × 8 = .12mm. = length of the object. It is not necessary to know the *actual* length of the divisions of the eye-piece scale. It is merely necessary that they be of equal length. Their valuations must be determined for each combination of objective, eye-piece, and tube length.

The unit of measurement adopted by microscopists is the micro-millimetre, or $\frac{1}{1000}$ part of a millimetre. It is sometimes represented by mmm; more commonly, and better, by the Greek Letter μ. An object which measures .001 of a millimetre in length is said to be 1 μ long; similarly .015 mm. = 15μ; etc.

To measure the magnification of a camera lucida drawing, place a stage micrometer on the stage with the microscope arranged for drawing. Place two dots on the drawing-paper to mark exactly the limits of the image of one division of the micrometer scale. With an accurate rule measure the distance between the dots. If a micrometer, with an English scale, divided in thousandths of an inch is used, and the image of one division measures one-fourth inch, the magnification is 250; similarly, if a micrometer, with a metric scale, divided in hundredths of a millimetre is used, and the image of one division measures one millimetre, the magnification is 100.

The Dissecting-Microscope. — This accessory is shown in Fig. 25. It is used mainly in the preparation of objects for examination under the compound microscope, or for permanent mounting. As the dissecting-microscope is a simple microscope, the image is not inverted, as is the case with the compound microscope. It is, therefore, easier, especially for a beginner, to work with a specimen under the dissecting-microscope.

Dissecting-needles are a necessary accompaniment of the instrument. They are easily prepared by thrusting ordinary sewing-needles, eye first, into wooden handles about the size and shape of a penholder.

In using the dissecting-microscope, the object to be studied is placed on the stage of the microscope in a watch glass, or other shallow dish with a clear glass bottom, or on a wide slip of glass, either in its natural state, or in a drop of water, or in a drop of some other fluid. It may then be conveniently dissected, treated with staining fluids and reagents, or otherwise specially prepared.

Some workers find the dissecting-microscope a necessity; others accustom themselves to the use of the compound microscope for this work, using a very low power. With practice the difficulties which are at first encountered

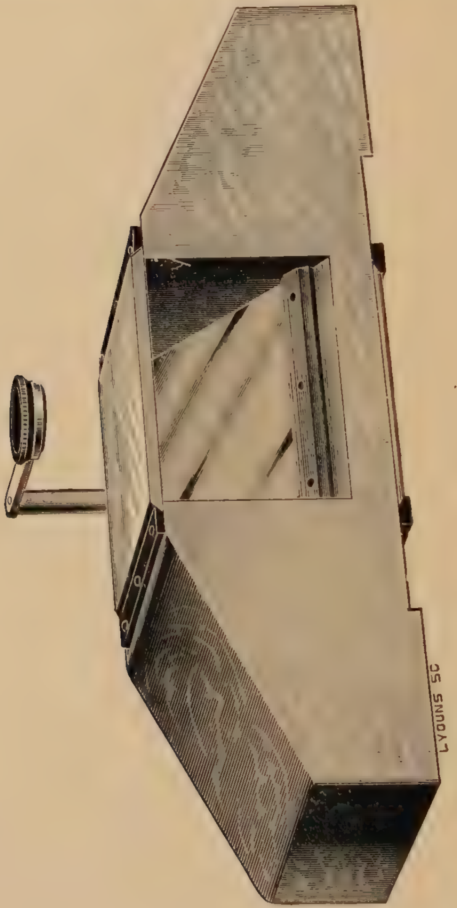


FIG. 25.

because of the inversion of the object are overcome, and there is no longer inconvenience.

The Turn-Table. — If one is to mount many objects, and particularly if he has an ambition to do neat work, a turn-table is a necessity. The instrument should be self-centring. It is the most convenient means of centring the object in mounting. The glass slip is placed on the turn-table, the prepared object placed on the centre, and the mounting medium and cover-glass applied before the slip is removed from the turn-table. The method of using the turn-table in finishing and ornamenting mounts is described in Chapter VI.

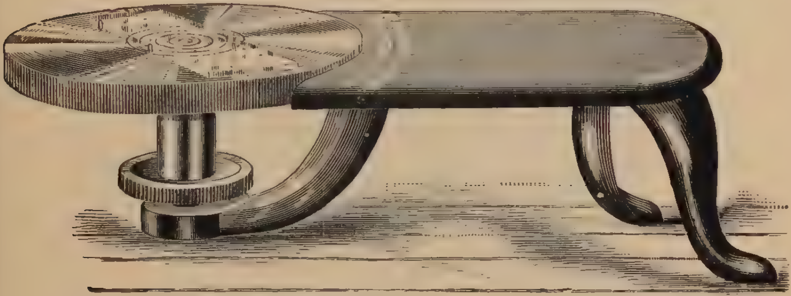


Fig. 26.

The Section-Cutter. — While, for purposes of studying fresh objects, sufficiently good sections may be made free hand by the method described in Chapter V., if many sections of uniform evenness and thickness are required, a section-cutter, or microtome, must be employed. There are many forms of both. Fig. 27 shows a convenient form of section-cutter to be clamped to the table. If the object is of a suitable degree of firmness to be cut without special preparation, as, for instance, many young plant stems, it is placed in the jaws of the clamp with its upper end even with the upper surface of the section-cutter. By means of the micrometer screw any desired measured thickness of section may be obtained. The sections are cut with a razor, ground flat on one side, held squarely on the glass plates on the top of section-cutter, and drawn across the specimen obliquely. In this instrument, one complete revolution of the micrometer screw raises the specimen $\frac{1}{50}$ of an inch. The head of the micrometer screw is graduated into ten divisions. If it be turned through one of these divisions, the object is raised $\frac{1}{500}$ of an inch. By turning the micrometer screw through a fraction of one of these divisions, sections of greater fineness may be cut.

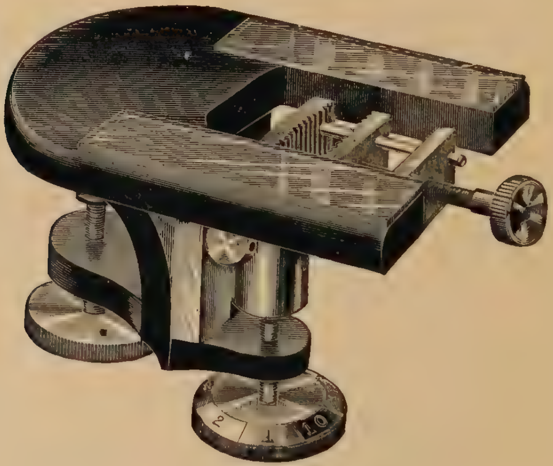


Fig. 27.

The Microtome. — Fig. 28 shows the laboratory microtome of the Bausch and Lomb Optical Company. The prepared specimen is placed in the jaws of the clamp, which may be adjusted at any height and at any angle by means of the adjustments, *H* and *G*. The lever, *J*, also furnishes means of raising and lowering the carriage, *F*, which carries with it *H*, *G*, and the clamp. The graduated disk, *K*, is mounted upon a delicately cut screw,

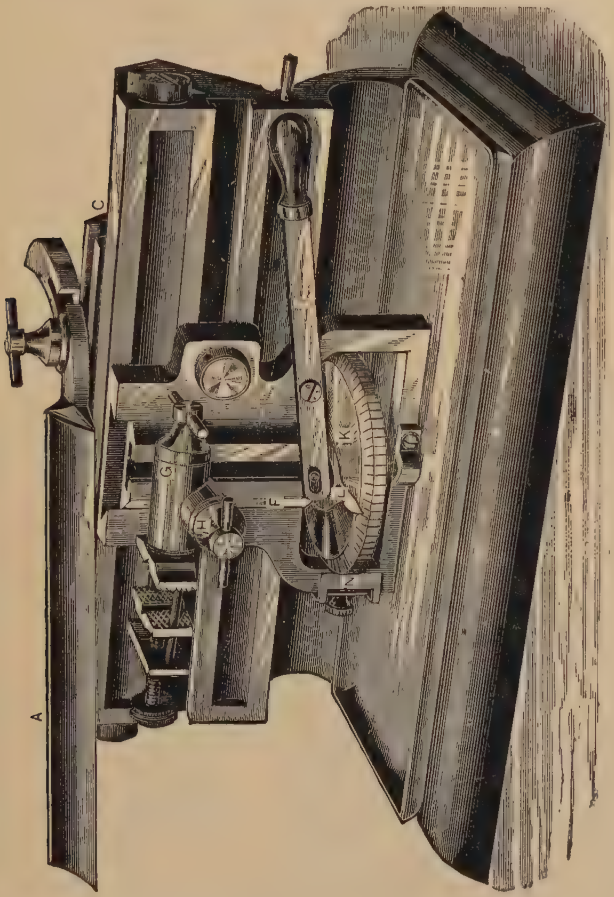


Fig. 28.

which, by turning *K*, may be brought up to support *F*. The pitch of the screw is $\frac{1}{50}$ of an inch. The disk has eighty divisions in its graduation. By turning the disk through one of these divisions, the clamp carrying the specimen is raised $\frac{1}{4000}$ of an inch. The amount which

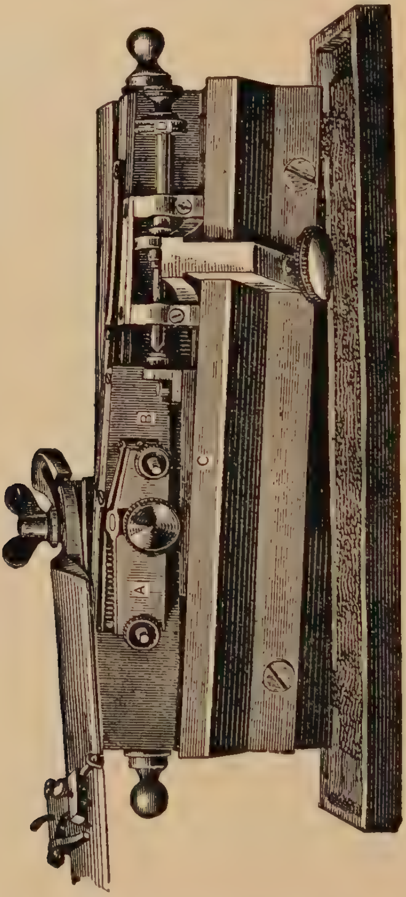


FIG. 29.

the disk, *K*, is turned is read by an index. A spring clip, *N*, may be adjusted to give a pronounced click as each division on the rim of the disk passes it.

The knife, *A*, is mounted on a block, *C*, which slides the entire length of the instrument. The knife may be placed so as to strike the specimen at any angle. It is usually adjusted to cut the specimen obliquely.

Thoma's microtome is shown in Fig. 29. It is a good example of the microtomes constructed on the principle of the inclined plane. The clamp, *A*, for holding the specimen to be cut is borne by a carriage, *B*, which moves on an inclined plane, *C*. This carriage is pushed forward by turning a micrometer screw, *D*, and thus the object may be raised through any desired minute distance.

For an example of the automatic microtomes, see Figs. 43 and 47.

Methods of preparing hard and soft tissues for cutting with the microtome and the section-cutter are given in subsequent chapters.

The Water-Bath and Oven. — In the process of preparing such objects as require imbedding in paraffin a water-bath is indispensable. A bath may, of course, be improvised. The form represented in Fig. 30 is very convenient. The vessel is capacious, holding a large volume of water. This is a great advantage, as the temperature is under much better control than when a small quantity of

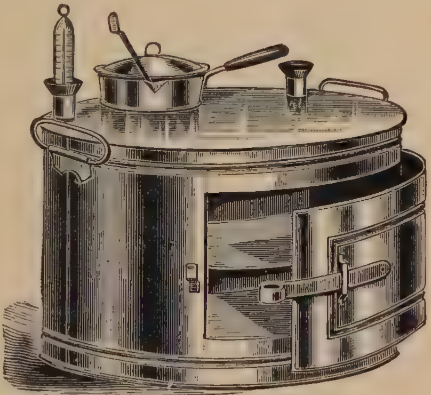


FIG. 30.

water is used. The paraffin cup sits in a compartment extending deep into the water. A thermometer indicates the temperature. The oven is useful in evaporating volatile reagents from a section, as in removing the clove oil from the collodion fixative used in fixing sections to the glass slip; also in hardening balsam mounts and some kinds of finishing cements. The bath is heated over a laboratory burner or an oil stove. A temperature of between 130° and 140° F. is suitable for most purposes for which the bath is used.

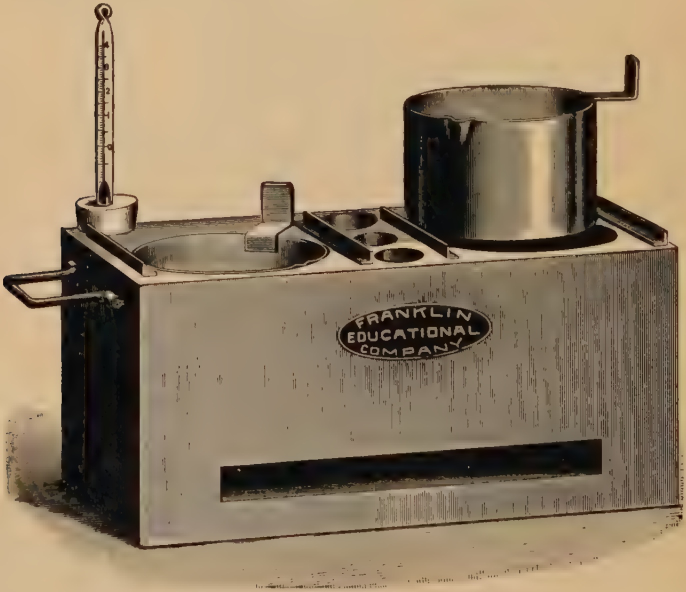


FIG. 31.

Fig. 31 shows a form of water-bath which is convenient in that two cups for the paraffin and places for test-tubes are provided.

CHAPTER III.

POLARIZED LIGHT AND THE POLARIZER.

THE elasticity of the ether in space is believed to be equal in all directions. The same is true of the ether in non-crystalline substances and in crystalline substances of the cubical system. The particles of ether are consequently free to vibrate equally in all directions. In other crystalline substances the elasticity of the ether is modified by the crystalline structure. In some crystals there is one axis or direction about which the molecules are arranged in a uniform manner; such crystals are said to be uniaxial. In other crystals there are two such axes. If a ray of light passes through a crystal along one of the axes, the ether particles are as free to move in one direction as in another; and, except that the light is retarded and to some extent absorbed, which is the case in all dense media, the ray passes through unmodified. If, however, the ray passes through a crystal in any other direction than along an optic axis, the particles of the ether are restricted in their motion in some directions more than in other directions. The result is that the wave movements are mechanically resolved into two sets of vibrations at right angles to each other, and still at right angles to the direction of motion of the ray; but as there is greater freedom of motion in one direction than in the other, the vibrations in this direction are longer than those in the other direction. The effect on the ray is that it is divided into two rays, each caused by vibrations in a single direction.

These rays are both refracted; but as the density of the crystal is different in different directions on account of the difference in structure, the rays are refracted to different degrees, so that they leave the crystal as two separate rays. In some crystals one of these rays is refracted like an ordinary ray of light, and is therefore called the ordinary ray. The other ray is differently refracted in different positions of the crystal, and is called the extraordinary ray. By the unaided eye these rays cannot be distinguished from ordinary light, but when examined by special means they are found to be quite different. Light when thus separated is said to be polarized.

The number of substances that exhibit double refraction is very large; in fact, it is the rule for crystalline substances other than those of the cubical system. Many organic substances also have such differences of structure in different directions that they act in a similar way upon light. The substance that exhibits this property in the highest degree, and the one that is of the most consequence to the microscopist from the fact that the polarizer and analyzer of the microscope are made of it, is Iceland spar, or the crystallized carbonate of lime. This is a uniaxial crystal, the axis joining the blunt angles. If an object is viewed through a crystal of Iceland spar in any other direction than that of the optic axis, it appears double, and if the spar is turned around, one of the images, that formed by

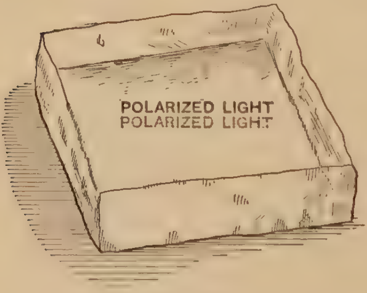


Fig. 32.

the extraordinary rays, revolves about the other. The ray which is most refracted is the ordinary ray. The index of refraction is 1.654. The index of refraction for the extraordinary ray varies from 1.654 to 1.483.

For use in optical instruments, it is desirable to obtain one of these polarized rays entirely apart from the other. This is very ingeniously done in the Nicol prism. In the construction of this prism, advantage is taken of the principle of total reflection, and of the fact that the index of refraction of Canada balsam (1.54) is less than the index of refraction of the ordinary ray, and more than the minimum index of the extraordinary ray. We have already seen in Chapter I. that when a ray of light, in passing from a dense into a rarer medium, strikes the surface at certain angles, it is totally reflected. A rhombohedron of Iceland spar, Fig. 33, three times as long as thick, is cut by a plane *AB*, joining the obtuse angles, the ends being cut off so as to form right angles with the dividing plane. The parts, after being polished, are cemented together with Canada balsam. A ray of light entering the prism from the direction *EF* is doubly refracted at *F*. The ordinary ray, *FK*, meets the surface of the balsam at an angle greater than the critical angle, and is totally reflected along the line *KL*, and thus thrown out of the field of view. The extraordinary ray, *FG*, passes straight through the prism, and after refraction on passing into the air, takes a course parallel to its course *EF* before entering the Iceland spar. By using the Nicol prism, then, we obtain a beam of

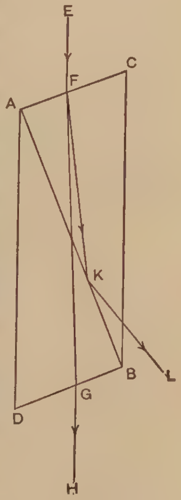


FIG. 33.

polarized light, or light caused by vibrations in a single direction at right angles to the direction in which the ray is travelling.

Two Nicol prisms appropriately mounted constitute the polarizer and analyzer of the microscope. One is mounted to be attached to the sub-stage, and is called the polarizer, Fig. 34. The other, the analyzer, Fig. 35, is screwed into the body of the tube, in the place usually taken by the objective, and the objective is screwed into the lower part of the mounting of the analyzer, so that the analyzer is between the objective and the body of the microscope. The analyzer is sometimes mounted in a double compartment box arranged to slide in and out in the body over the objective. In one position of the box the light passes through the analyzer. In the other position the light passes through an empty opening. This is a very convenient way of mounting. The analyzer is also mounted to be placed over the eye-piece in some instruments. The only conditions that must be fulfilled are that objects to be examined by polarized light must be

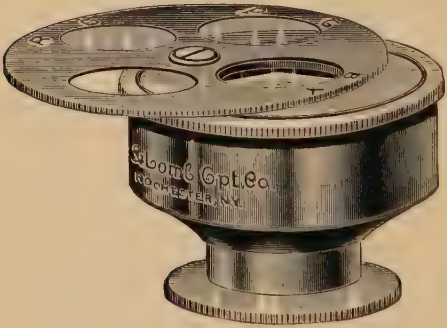


FIG. 34.

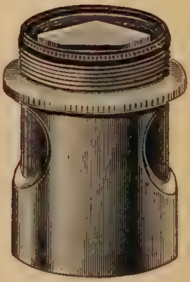


FIG. 35.

placed between the polarizer and analyzer, and the Nicols must be so placed that the whole of the light can pass through them.

Either the polarizer or analyzer is mounted so as to be rotated about the optical axis of the microscope. In the better grades of microscopes, the mounting of the rotating Nicol has a graduated circumference. When the rotating Nicol is in the same position as the fixed Nicol, the axes of the Nicols are said to be parallel. When the rotating Nicol has been turned through an angle of 90°, the axes are said to be crossed. The effects produced are precisely the same whether it be the polarizer or the analyzer that is rotated. For purposes of explanation, it is less confusing to consider that the analyzer is turned. In the following pages, then, the analyzer will be understood to be the rotating Nicol.

Place the polarizer and analyzer on the microscope, using a low-power objective and eye-piece, and turn the rotating Nicol until the field is well lighted. Observe the reading of the graduated circumference, and turn the Nicol through 90°. The field is now dark. Turn the Nicol through another 90°, and the field is again light. Turn it another 90°, and we have darkness again. Turn it still another 90°, and we have completed the circle and come back to the original position of light.

We will trace a ray of light in its passage from the mirror through the Nicols. As we have seen, the ray is doubly refracted by the Nicol, the ordinary ray being thrown out of the field of view, and the extraordinary ray, which is caused by vibrations in a single direction at right angles to the direction in which the light is travelling, is alone left to pass on. The vibrations which produce this ray are in a definite plane, one which permits them to pass through the Nicol prism in planes lying in a particular direction. If the analyzer is in exactly the same position as the polarizer, or if it is turned 180° from the position of the polarizer, these particular planes lie in the same direction in both polarizer and analyzer. The vibrations can pass, and the field is light. Turn either Nicol from this position, and the particular planes which allow the vibrations to pass do not correspond in direction in the two Nicols. As the rotating Nicol is turned from the position parallel with the other Nicol, the field gradually grows dark. This is due to the fact that the vibrations strike the second Nicol at an acute angle with its planes of vibration, and are mechanically resolved into two sets of rays at right angles to each other. The rays of one set are in the planes of vibration of the second Nicol, and pass, lighting the field to some extent; the rays of the other set are at right angles to the planes of vibration in the second Nicol, and are suppressed. As the angle at which the rays strike the planes of vibration of the analyzer increases in size, the resolution of the rays becomes less and less, until, when the analyzer has been turned 90°, the rays strike the planes of vibration of the analyzer at right angles. There is now no resolution of the rays, the light cannot pass at all, and the field is dark. Continue to turn the analyzer. The rays now strike its planes of vibration at an acute angle, and are resolved into two sets. At first the amount of the resolution is small, but it increases until the analyzer is turned 90° from the position of darkness. The rays now strike the planes of vibration of the analyzer at an angle of 0°, or parallel to these planes. They are wholly transmitted, and the field is light. Note that this is the position 180° from the first position of light. Again continue to turn the analyzer, and the field gradually grows dark for the same reasons as in the first case.

Colors in Thin Plates. — Cross the Nicols so as to produce a dark field. Place on the stage of the microscope a thin piece of mica, and slowly revolve it. Four positions of the mica, or two directions at right angles to each other, will be found in which the field remains dark. For all other positions of the mica, the field will be more or less lighted, and if the thickness of the mica plate is right, the light will be colored. The brightest colors are seen when the mica is turned 45° from the position of darkness.

Mica is a doubly refracting substance. When in the positions of darkness, one set of the planes of vibration by which light can pass is parallel with the planes of the polarizer, and the other set with the planes of vibration of the analyzer. It is as though one set of these planes of vibration in the mica were a continuation of the same planes in the polarizer, and the other set a part of the corresponding planes of the analyzer. The light is not further modified, and the field remains dark.

In any other position of the mica plate, the ray coming from the lower Nicol, striking against the mica, is doubly refracted, and passes out of the mica as two rays in directions parallel to each other. As in the case of Iceland spar, one of these rays is more refracted than the other, and must pass through a greater thickness of mica before it emerges. It is consequently more retarded than the other ray during the passage through the dense mica, and comes out a short distance behind the other. If, now, the thickness of the thin plate of mica is right, the retardation may be of such an amount that if the two rays can be brought together so as to interfere, the waves resulting from the interference will be of the right length to produce the sensation of color. But observe that the rays are each caused by vibrations in a single direction, and the directions for the two rays are at right angles to each other. There can be no interference unless the rays can be brought into the same plane. This is what actually happens in the analyzer. Suppose the mica to be in one of the positions which give the brightest color. The two sets of vibration at right angles to each other, coming from the mica plate, strike the analyzer at an angle of 45° with its planes of vibration. Each of the two rays is mechanically resolved into two sets of vibrations at right angles to each other, one set of vibrations from each of the original rays lying in the planes of vibration of the analyzer, the other set from each lying at right angles to the planes of the analyzer. The latter are suppressed; the former pass, and if one is behind the other by half of the wave length of any of the colors composing the white light of which the rays are made up, that color is suppressed through interference, and the color of the transmitted rays is complementary to the suppressed color. The particular color produced depends on the thickness of the mica plate, for, as is well known, the waves that produce the sensation of red light are nearly twice as long as those that produce the sensation of violet light, and the other colors are produced by waves of lengths intermediate between these extremes. Different thicknesses of mica, therefore, cause interference of the waves of different colors, and the transmitted complementary colors are different. If, however, the mica is quite thick, the retardation is such as to suppress by interference portions of the several colors of which the white light is composed, so that the effect is simply to reduce the intensity of the white light that is transmitted.

Without changing the positions of the polarizer and analyzer, place pieces of mica of different thicknesses upon the stage, and in each case turn the piece until the brightest color is produced. It is at once seen that, within certain limits of thickness, each different piece produces a different color, but that pieces beyond a certain thickness do not cause color.

Complementary Colors. — Cross the Nicols, place a plate of mica on the stage, and turn it until the field is dark. If the microscope has a graduated revolving stage, the next step is easy; if not, the measurements may be taken with sufficient accuracy with a protractor. Revolve the mica until the field is brightest. It will be found, by accurate observation and measurement, that the mica has been turned through 45°. The two rays coming from the mica now strike the analyzer so that the vibrations of each form an angle of 45° with the planes of the analyzer. As we have before seen, each vibration is mechanically resolved into two at right angles with each other, one set of vibrations from each of the original rays lying at right angles with the planes of vibration of the analyzer, and so not able to pass, the other set of vibrations from each of the original rays lying in the same planes with the planes of vibration of the analyzer. The latter pairs of vibrations pass, interfere, and produce waves of the right length to cause the sensation of color. Observe what happens when the analyzer is turned through 90° from this position. The pairs of vibrations, which before could pass, now strike the analyzer at right angles to its planes of vibration, while the pairs, which before were suppressed, now lie in the same planes with the planes of vibration of the analyzer, pass, interfere, and cause a color.

Observe that the sum-total of the vibrations which caused the last pairs of rays, plus the total vibrations which caused the former pairs, originally produced white light. We should, therefore, expect that the color produced in the last instance, added to the color produced in the first, would produce white light; in other words, that the colors would be complementary. We find that this is the case.

If we turn the analyzer through another 90°, its planes of vibration lie in the same directions as in the first instance, and the same color is seen. Turn the analyzer through another 90°, and the complementary color must again result.

The Dark Cross. — Cross the Nicols, and place upon the stage a slide of sulphonal crystals prepared by the method described in Chapter X. The light is more or less restored, and black crosses, the arms at right angles to each other, are seen. Bring the point at which the arms of one of these crosses intersect to the centre of the field, and revolve the specimen on the stage. The cross will be seen to be stationary. It shows the position of the planes of vibration in the Nicols, one arm of the cross lying in the planes of the polarizer, the other in the planes of the analyzer. It appears because there is no polarization in these two directions, the light being simply absorbed. Other portions of the crystal are colored because there is polarization in all other directions.

Turn the analyzer 90°, and a white cross takes the place of the black one, the colors of the intervening spaces at the same time changing to their complementaries, so that there is an appearance of a revolving cross changing color as it revolves. There is still no polarization in these two directions, but the light is now transmitted.

A full explanation of these phenomena would involve a discussion of elliptical polarization, which would require too much space for a proper presentation here. Suffice it to say that these appearances are similar to the phenomena produced by uniaxial crystals cut perpendicular to their optic axes, and viewed in convergent polarized light. In the crystals of sulphonal the point of intersection of the arms of the cross is a point on the axis of the crystal. From this point the crystallization proceeded laterally in all directions. This may easily be verified by watching the formation of the crystals when the slide is prepared. These crystals are, then, similar in structure to sections of uniaxial crystals cut perpendicular to the principal axis. Such sections of quartz, emerald, mica, and other uniaxial crystals show the cross when viewed in convergent polarized light. Crystals of salicin and other chemical salts which form from centres, as do the sulphonal crystals, on account of the peculiar arrangement of their molecules around the axis, show the cross in plane polarized light. Starches, for a similar reason, show a more or less distorted cross, very characteristic and interesting, radiating from the hilum.

Uses of Polarized Light. — As has already been seen, the particular color produced by the interposition of a thin film depends upon the thickness of the film. If the film is of varying thickness, or of varying composition, different colors are produced simultaneously. In the case of many chemical crystals the slight differences in thickness give rise to most beautiful combinations of colors, rendered still more striking by the changes to complementary colors as the analyzer is turned. Many crystals that are almost, if not quite, invisible in ordinary light are made plainly visible by using polarized light; in some cases revealing most delicate and beautiful forms. It is hardly possible to use too strong language in describing the fascination felt in watching the process of crystallization under polarized light, or even in examining crystals already formed and permanently mounted.

In the case of rock sections the different minerals of which the rock is composed affect the light differently, and the different effects produced are important aids in distinguishing and determining the composition of the rock.

So, too, in the case of many common objects, there are sufficient differences in structure to polarize the light in greater or less degree. The structure of the object is usually made much plainer in some positions of the analyzer. Polarized light is, therefore, a valuable means of effecting special illumination of some classes of objects. It is always well to try polarized light on any very transparent object. The results thus obtained are in many cases equally surprising and interesting.

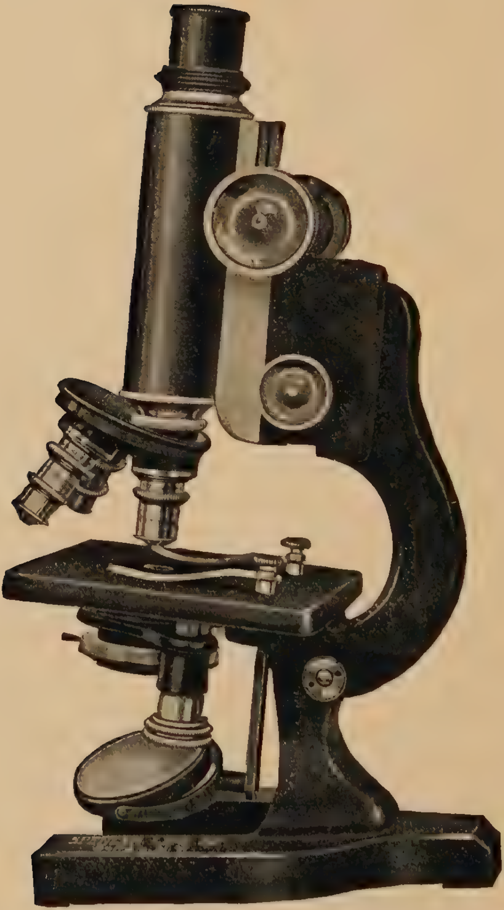
A few of the large number of polarizing substances are the following: thin and transparent cellular tissue of plants; plant hairs, which often yield most striking effects; starches; the silicious cuticle of plants; fibres of cotton, wool, and hemp; crystalline deposits in plant tissues and sections; muscular tissues of animals; animal hairs; sections of bone, horn, and similar substances; all crystalline substances except those of the cubical system. It would not be possible, nor is it desirable, to give a complete list. Even when an object does not distinctly polarize, the modification of the light effected by turning the analyzer aids materially in bringing out the less distinct portions of the structure.

Substances which are not of the right thickness of themselves to polarize often give good effects when a film of selenite or mica is used with them to increase the thickness. Such a film should form a part of the polarizing outfit. It may be introduced anywhere between the polarizer and analyzer. A single film is sometimes mounted in the fitting of the polarizer, or several films of different thicknesses are so mounted on the polarizer fitting that any one may be interposed at will, as is shown in Fig. 34. The film is sometimes mounted in a brass plate to be laid upon the stage. It is then called a selenite stage. Sometimes it is mounted in a small metal plate to be inserted in an opening made for it in the body of the microscope just above the objective, but, of course, below the analyzer.

A well-selected mica film, mounted in Canada balsam on an ordinary glass slip, and covered with a thin cover-glass to prevent its becoming scratched, serves the purpose well.

Single-Refracting and Double-Refracting Objects. — It is evident from what has already been said that polarized light affords means of determining whether a substance is singly refractive or doubly refractive. If a singly refractive substance, as, for instance, a piece of ordinary glass, or a crystal belonging to the cubical system, as crystals of potassium iodide, potassium bromide, or common salt, is placed upon the stage with the Nicols crossed, the field remains dark, nor are color effects produced when the analyzer is turned. Such substances are said to be optically isotropic or monorefringent.

If a doubly refractive substance, as, for instance, any chemical crystal not belonging to the cubical system, or any other of the substances already mentioned as polarizing substances, is placed upon the stage, the Nicols being crossed, the light is restored and color effects are produced, when the analyzer is turned. Such substances are said to be birefringent or anisotropic.



Spencer Microscope No. 25.

Fig. 36.

CHAPTER IV.

INSTRUCTIVE PRACTICE IN MANIPULATION.

The beginner will do well to make a careful study of the microscope before attempting practical work. With this end in view, he may put himself through a course of training in microscopical gymnastics. The knowledge gained will be ample reward for the trouble. One or two slides of diatoms, to be purchased at twenty-five cents each of various dealers, will afford means for very instructive study of the instrument. A slide of the diatoms called *Arachnoidiscus Ehrenbergii* and one of *Pleurosigma angulatum* will serve the purpose. In the lack of these slides the scales of a moth, obtained by pressing the wing of a moth to a cover-glass, or a little potato starch dusted on a cover, may be used. Invert the cover on a glass slip.

Place the slide of *Arachnoidiscus Ehrenbergii* on the stage of the microscope and focus sharply upon it, using the 6 x eye-piece and the 16 mm. objective. In focussing, do not at first look through the instrument, but place the eye in position to watch the approach of the objective to the glass slip on the stage, and slowly move the body of the microscope down by means of the coarse adjustment until the front lens of the objective is as near its working distance from the glass slip as can be estimated; in this case about one-fifth of an inch. Now look through the instrument, and the outlines of the object should be more or less visible if the object is properly centred on the stage. If nothing is seen, move the glass slip about to centre the object more perfectly. If it is now necessary to use the coarse adjustment to change the focus, focus up only. If the object is not thus brought into view, turn the body down again with the coarse adjustment while watching the approach of the objective to the object as before. When the object is brought into view, focus up and down with the fine adjustment to obtain a more perfect image. Recall what was stated in Chapter I. about the influence of the thickness of the cover-glass on the clearness of the image. If the thickness of the cover-glass is known, adjust the draw-tube to the required length. If the thickness of the cover-glass is not known, after getting the sharpest image possible with the draw-tube at the standard length, try the effect of longer and shorter lengths until the image is clearest.

See that the field is properly lighted. Direct sunlight must not be used. The diffused sunlight from a north window, or the light reflected from a cloud or white wall, is regarded as the best light. The light from an ordinary kerosene lamp is a very suitable artificial light.

Place the mirror in central position, and use first the plane and then the concave side. Use each of the openings in the diaphragm in succession with each mirror. Focus up and down occasionally with the fine adjustment. Practise these manipulations until the arrangements are found which show the markings on the diatom most perfectly. It will be found that the best effects are obtained when the field is lighted with a subdued light that is very pleasant and comfortable for the eye. A glare of light should always be avoided. The light should be sufficient, but not too intense. If the light is thus properly regulated, and suitable precautions are exercised not to use the eyes to the point of fatigue, the eyes will be strengthened rather than injured by microscopical work. Just as judicious exercise strengthens the body, so judicious use of the eyes improves them. If one makes a practice of reading in the glare of the sun or other light, his eyes will soon be injured. So too, if he uses the microscope with glaring light, his eyes will be injured. The correct modulation of the light is a matter of very great importance, and one that is too often overlooked even by those who have had considerable experience with the microscope. The light from the concave mirror is more intense than that from the plane mirror, and is better adapted to the higher power objectives and eyepieces, which absorb more light than do the lower powers. In using the concave mirror, slide it up and down on the mirror bar until a bright circle of light from half an inch to an inch in diameter is projected upon the slide. This circle of light is best seen when the slide is shaded from other light.

After noting the effects obtained from each of the above arrangements, move the mirror about 10° to the side away from the light, adjust it to the light, and again go through the same practice manipulations, carefully observing differences of effects. Then move the mirror another 10° to the side, and again repeat the manipulations. Continue to move the mirror to one side 10° at a time, and to repeat the above manipulations as long as good results can be obtained. Having observed the effects for each position of the mirror with the draw-tube closed, place the draw-tube at different lengths. Notice the slight change of focus necessary and the effect on the image. Determine what length of draw-tube gives the best results.

Remove the 6 x eye-piece and put in its place the 10 x eye-piece, and practise the manipulations again. When some degree of skill has been acquired in manipulation, the 4 mm. objective may be substituted for the 16 mm., and the slide of *Pleurosigma angulatum* for the *Arachnoidiscus Ehrenbergii* as a more suitable object for this objective. In focussing the 4 mm. objective the precaution to watch it as it is brought to its proper working distance, about $\frac{1}{40}$ of an inch from the slide, must be carefully observed. On no account allow the front lens to come in contact with the cover-glass, or both objective and cover-glass may be injured. With both the 6 x and the 10 x eye-pieces repeat the manipulations described above until the best results possible are obtained.

Patient practice of these manipulations at intervals for a few weeks will give one a knowledge of the instrument, and an ability to handle it in practical work that cannot be gained by years of heedless use.

Examination of Opaque Objects. — Place a glass slip upon the stage, and on it any opaque object, as, for instance, seeds of grasses or flowers, a plant leaf, grains of sand, or an insect. If the mirror can be swung above the stage, arrange the concave mirror so as to focus the light upon the object. This should be done roughly before looking into the microscope. With the coarse adjustment move the objective to about its working distance. Then looking at the object through the microscope, focus up and down with the coarse or fine adjustment, as occasion may require, always remembering to guard against bringing the objective too near the slide or specimen. Change the position of the mirror to illuminate the object better. If the mirror cannot be used thus, a bull's-eye condenser may be used to focus the light upon the object, the mirror being put in such position that light reflected from it will not interfere with the illumination.

Air Bubbles. — Many curious and ridiculous blunders have been made from misinterpretations of the appearances of air bubbles. To guard against such errors, air bubbles should be made the object of special study. A drop of saliva, or soapsuds, or of mucilage well beaten up on a slide with the point of a knife blade, have been suggested as suitable means of easily obtaining the bubbles for study. Study the bubbles both without and with a cover-glass, which should be placed lightly over the mass. Focus up and down, using both central and oblique light. Once studied in this way, the air bubble will ever after easily be recognized. Observe that air bubbles afford a most excellent means of determining when the light is central.

Oil Globules. — Oil globules have also led to errors of interpretation. They are liable to occur in preparations of animal or vegetable tissues. To form them for study, beat up a small drop of any oil with a drop of water on a slide, cover lightly, and study them in the same manner as the air bubbles.

Fibres and Hairs. — Fibres of cotton, wool, silk, and hemp, and hairs of different kinds, are liable to find their way into preparations from the air of the room, or from lack of care during the processes of preparation. It is well to make a special study of these and similar objects. Examine them dry, also in water or glycerine, or in some oily medium, as oil of turpentine, or oil of cloves, covering with a cover-glass. Examine in a similar manner any other objects which may suggest themselves as likely to find their way adventitiously into a microscopical preparation.

Currents and the Brownian Movement. — Grind up a little solid carmine, or any very finely powdered mineral substance, in a drop of water on a glass slip, cover with a cover-glass, and place the slip on the stage of the microscope. Incline the stage, and watch the current movement of the particles. Then place the stage in the horizontal position, and wait a few minutes for the currents to cease. A peculiar, jerky motion of the finer particles may now be observed as they dart about among the larger masses. This is the so-called Brownian movement, or Pedesis. The study of these phenomena may prevent serious errors of interpretation.

Practice with Immersion Objectives. — On beginning to use an immersion objective, practice work is of especial importance. The slide of *Pleurosigma angulatum* is still a good object to use, as the 4 mm. objective has left much unseen. With this slide on the stage, place a small drop of water, if the objective is a water-immersion objective, or of homogeneous immersion fluid, if the objective is a homogeneous immersion objective, on the front lens, or on the centre of the slide, the microscope being placed in the upright position. Watching the objective, run it down by means of the coarse adjustment in the manner previously described, until the drop of fluid forms a connection between the front lens and the cover-glass. The drop should be just large enough to fill the space between the lens and the cover-glass. After the connection is made, the microscope may be inclined, if desired. If a non-adjustable objective is used, and the thickness of the cover-glass is known, the draw-tube should be adjusted to give the correct tube length according to the cover-glass gauge. When the tube length can thus easily be obtained, much has been gained. We have now simply to light the field well by means of the sub-stage condenser, remembering that as much depends upon correctly focussing the condenser as on focussing the objective, and obtain the correct focus of the objective by means of the fine adjustment. One should remember always to focus up and not down, or both the cover-glass and objective will be endangered.

If the objective has collar adjustment, and the thickness of the cover-glass is known, the collar may be set at once at the proper mark, remembering that the objective should be set at the medium point for cover-glasses of the standard thickness adopted by the makers of the objective. If the cover-glass is thinner than the standard thickness, the milled collar should be turned towards the lowest point. If the cover-glass is thicker than standard thickness, the milled collar should be turned towards the highest point, the amount of the turning being estimated by the amount of deviation from the standard thickness of cover-glass.

If the thickness of the cover-glass is not known, the collar should be set at the highest point, that is, for thick covers, when the connection is first made with the cover-glass. Now with one hand on the milled ring of the collar adjustment, and the other on the fine adjustment, and while looking into the microscope, turn the collar adjustment through part of one of its divisions, and gently focus up and down with the fine adjustment. Repeat this manipulation until the most perfect image attainable has been obtained, remembering to test the focussing of the sub-stage condenser from time to time by moving it up and down.

After obtaining the best effects possible with central light, oblique light should be used. If the sub-stage condenser is provided with a diaphragm, make the diaphragm eccentric; that is, move it a little to one side. After noting the effects, by successive steps make the diaphragm more and more eccentric as long as good results can be obtained. Then, in like manner, move the diaphragm in the opposite direction. If there is no diaphragm, obtain oblique light by means of the mirror by moving it away from the light a few degrees at a time in the same manner as when using the low power objectives.

It cannot be stated too emphatically that a large amount of this practice manipulation should precede any attempts to make practical use of immersion objectives. The ability to bring out the full power of these objectives can be attained only by patient perseverance and the intelligent study of the effects produced by manipulation. It hardly seems necessary to waste time in describing the results which should be obtained. The observer will have very little doubt about his success when he obtains clear-cut images, and he should not be satisfied until he does this.

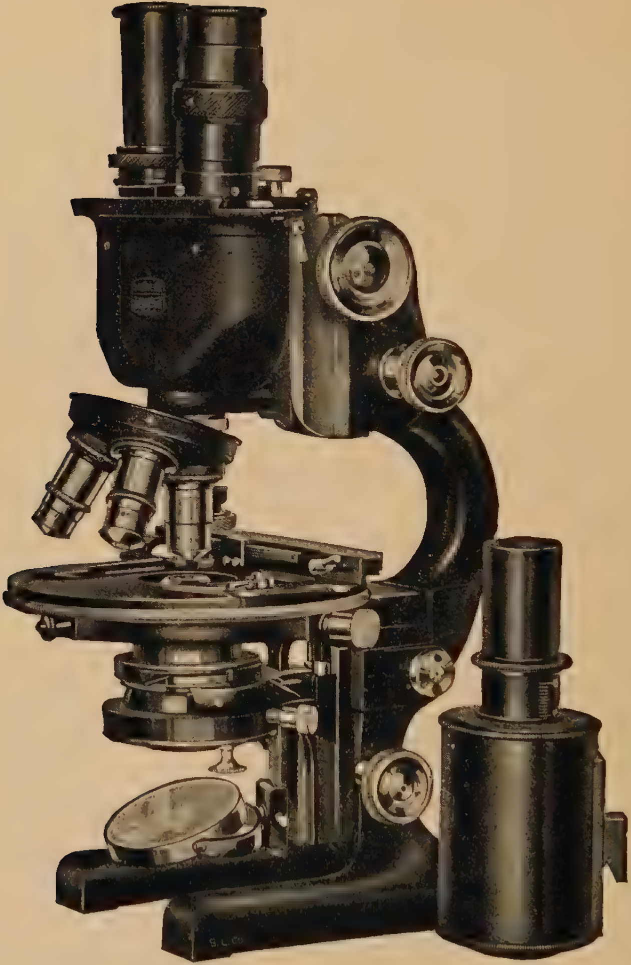
CHAPTER V.

METHODS OF STUDYING FRESH OBJECTS.

THE microscope is but an aid to the eye, and the eye an aid to the brain behind it. Something more than the mere collection of objects and multiplying the number of slides in a cabinet should be the aim of every owner of a microscope. The highest use of the instrument is in following out definite courses of investigation. In the pursuit of such investigations the examination of a very large number of different specimens is necessary to obtain clear ideas of structure, and give the brain material with which to work. It would not be possible, nor would it be desirable, permanently to preserve all the specimens examined. The methods of working will of course differ with different lines of investigation.

It is not the purpose here to furnish directions and descriptions for detailed and comprehensive work in any department. Volumes would be required for this, and excellent manuals already exist for the different departments of investigation. It will be convenient for the purposes of this chapter to consider some methods of working with vegetable and animal specimens, and to illustrate the methods by particular descriptions in a few cases. For methods of preparing vegetable and animal specimens for permanent mounting the reader is referred to Chapters VIII. and IX.

In the investigation of these specimens, a razor ground flat on one side, a supply of glass slips and cover-glasses,



SPENCER MONOBJECTIVE MICROSCOPE.

FIG. 37.

some dissecting-needles, and various inexpensive chemicals and stains, formulas for which will be found in the Appendix, are necessary. Glass slips three inches by one and a half are to be preferred for this purpose to the ordinary slips, as they present a broader surface to hold the reagents used. For many of the processes a dissecting-microscope is a great convenience. This is most needed when many dissections are to be made. If this instrument is not at command, use the lowest power of the compound microscope.

The microscope is best kept in the upright position to prevent the specimen from slipping out of the field of view, to prevent currents due to gravity in the fluid media employed, and to prevent the reagents from injuring the microscope. Neatness in all operations is the mark of a skilful workman. In case any of the reagents used accidentally come in contact with the microscope, they should immediately be removed. The reagents should not be allowed to flow over the upper surface of the cover-glass, as they are liable to come in contact with the front lens of the objective. Especial pains should be taken to prevent this, and in case they do touch the objective, it should immediately be cleaned with great care.

In these practical studies air bubbles and oil globules are very sure to be met with, and it may be well to recall or repeat the studies already made of these objects.

Vegetable Specimens.

For purposes of study vegetable substances are usually examined in water. Some substances require no special preparation. Any of the minute and transparent plants, either those growing on land or those growing in the water, may be placed on the centre of a glass slip in a small drop of water, a cover-glass laid over the specimen, and the examination made. Care should be exercised not to use so large a drop of water that it will run out from under the cover-glass. Enough only should be used to fill the space between the cover-glass and the slip. If more is added, it is liable to run on to the microscope. In any case the work is slovenly. An excess may be removed by touching it with a piece of blotting paper or of filter paper.

Microscopic Water Plants. — The light green stringy masses found floating in the water or attached to submerged objects in ponds and ditches in summer afford good material for examination. These plants belong to the family of Zygnemaceæ. They are unicellular, but the cells are joined in large numbers so that the plant appears to be multicellular. It is easily recognized by its slippery feeling when a mass of it is taken in the hand, and from the fact that each filament is without branches. In young cells the greenish yellow chlorophyll granules are quite evenly distributed throughout the cell. At more advanced stages the chlorophyll collects in some species in a line running lengthwise through the centre of the cell, often terminating in star-shaped or globular knobs, so that each cell contains a dumb-bell-like figure. These knobs are sometimes left isolated by the breaking down of the connecting line of chlorophyll granules.

In other cases the chlorophyll collects into spiral bands, differing in number in different species, the bands giving these species their name, *Spirogyra*. In favorable cases conjugation between the cells of two adjacent-lying filaments affords opportunity for much interesting study. June or July is the most favorable time to observe the conjugation. From this yoking together of the cells in conjugation the whole family takes its name, Zygnemaceæ. There are many varieties in the family, any one being an interesting object for study.

In examining this plant, observe that the cells are all alike, and, being joined end to end, form filaments of great length, but of uniform diameter throughout. As the plant approaches maturity, the bright yellow-green changes to a dirty brown. When at this stage, it is in a particularly favorable condition to exhibit some of its distinguishing features.

Attached to the cells of the *Spirogyra* are frequently found numerous spherical, or nearly spherical, bodies of a reddish color. They are the *Vampyrella spirogyræ*, belonging to the Monerozoa, one of the lowest types of animal life. They have attacked the *Spirogyra* to feed on its chlorophyll.

Yeast. — Yeast, *Saccharomyces cerevisiæ*, is another plant which furnishes interesting material for examination. Dissolve some sugar in water, and add a little yeast. When it is seen that the yeast is working, place a drop on a glass slip without a cover-glass, and examine with a low power. Then cover, and examine with a power of four or five hundred diameters. The yeast is seen to be made up of very transparent spherical and oblong cells of the same kind, but of varying sizes. Some will no doubt be seen with minute cells attached, showing the characteristic mode of propagation by budding.

Other Studies. — In a similar manner, any other substances which are sufficiently thin to allow light to pass through them may be examined. Pollen grains are thus easily examined, and are very attractive objects. Their forms are almost infinite in number and variety. The spores of ferns and mosses, and the numerous varieties of fungi, afford abundant material for examination. Of the fungi, mention may be made of the green mould which forms on bread, old leather, and numerous other substances. It is the *Penicillium glaucum*. Another mould, the *Mucor stolonifer*, is easily grown on a piece of bread kept moist under a bell-jar or tumbler for two or three days. It will be recognized by its fine, thread-like stalks, each having a round head, and by the stolons connecting the stalks. In examining either of these fungi, treat different specimens with hæmatoxylin and carmine stains, and with iodine.

The epidermis of leaves and stems may in many cases be scraped off with a knife, or torn off with forceps, and without further preparation be placed under the microscope in a drop of water. The epidermal cells, stomata, and hairs, are thus readily obtained for examination from many plants.

Maceration and subsequent teasing or tearing the specimen to pieces by means of dissecting-needles is a very important means of reaching the minute internal structure of plants. The maceration may be effected by soaking the specimen for some time, or by boiling it a few minutes, in water. It may be effected more quickly and thoroughly by treating with a warm, strong solution of caustic potash, or by placing the specimen in a test-tube with a few crystals of potassium chlorate and enough strong nitric acid to cover them (Schultze's Macerating Mixture). Strong hydrochloric acid is also a good macerating fluid. After the action has continued for a short time, the specimen should be washed thoroughly in water, transferred to a glass slip, and torn to pieces with the dissecting-needles under the dissecting-microscope or a low power of the compound microscope. Spiral and scalariform vessels are easily obtained by this process from the leaf stalks of rhubarb and of ferns, the stem of banana, and other sources. The cell structure of some tissues is also revealed by this treatment much better in some respects than can be done by sections.

Vegetable Sections. — To obtain sections of vegetable specimens, a piece is held firmly in the left hand between the thumb and second joint of the forefinger, the blade of the razor is rested on the second joint of the forefinger as a guide, and drawn towards the person, resting both hands against the breast to steady them. The thumb of the left hand should be kept depressed to guard against injury. Keep the specimen and the blade of the razor wet with water, and when the section is cut, dip the razor in a glass of water to float the section off. Small or soft objects may be held between pieces of pith, carrot, or potato. Hard specimens may be made soft enough to cut by boiling for a few minutes in water. The hard woods yield to this treatment. For purposes of study it is not always necessary that large, even sections be cut. Indeed, an uneven section is sometimes more valuable than an even one for this use. Some parts near the edges will show extreme thinness, while other parts often give a better idea of the relations of the cells to one another. Ordinarily the rule for thinness is to cut to the thickness of one row of cells. It is best to cut quite a number of sections of each specimen.

For study, a section should be transferred from the glass of water to a glass slip with a small drop of water. A camel's-hair brush is the best section-lifter. The section is covered with a cover-glass and is ready for examination. While water is ordinarily the best medium for the examination of vegetable substances, there are some cases where it produces an undesirable change in the tissues of the specimen, causing the cell contents or the cell walls to swell. Other reagents then become desirable. Strong alcohol, by extracting water, causes a contraction of the cell wall and contents. More dilute alcohol is, in many cases, a suitable medium to maintain the tissue in its normal condition.

It is often desirable to watch the changes which the specimen undergoes under the action of a reagent. It may be desired, for instance, to remove water from the specimen and introduce alcohol. This is best done without removing the specimen from the slide, or even removing the cover-glass from the specimen. The slide still remaining under the microscope, by means of a pipette (a glass tube drawn out to a fine point) hold a little alcohol near one side of the cover-glass, and at the opposite side hold a piece of blotting paper, or better, good filter paper. The water is drawn out from under the cover-glass by capillary attraction, and the alcohol takes its place. By repeating the operation several times the water is entirely removed and alcohol substituted. In the same way any reagent or stain may be introduced, or the specimen may be washed free of a reagent by means of water.

It often happens that a specimen is too opaque to be well studied, even when the section is very thin. In such cases glycerine is a valuable medium for the examination. If the specimen is already mounted in water, the water should be removed and the glycerine introduced in the manner just described. The changes which the specimen undergoes during the process afford excellent opportunities for studying the object. Opaqueness may also be remedied by bleaching the specimen before it is mounted. Sodium or calcium hypochlorite is a good bleaching agent.

A considerable volume of the fluid should be used, and the specimen should remain in it for some hours. If it is desired to render the specimen more transparent during the progress of the examination, a moderately strong solution of caustic potash may be run under the cover-glass. The first effect of the caustic potash in some cases is to turn the section black. This may be remedied by washing out the caustic potash with water, and running under a very weak solution of hydrochloric acid. The specimen soon becomes transparent. The acid should now be washed out with water. In the more obstinate cases it may be necessary to repeat the treatment with caustic potash several times before the desired effect is produced.

In similar ways the changes which the specimen undergoes when treated with different chemical solutions may be studied. It is usually best to add the reagent in small amounts at first, and to watch the changes as the strength is increased. Any of the staining solutions for which formulas are given in the Appendix may be thus employed. The use of different stains on the same section, or on different sections of the same specimen, tends to reveal varied points of structure. The study of fresh sections in this way is a valuable preparation for the correct use of stains for permanent mounts.

Starch. — Under the microscope starch granules show a distinct point, usually situated near one end, called the hilum, and an appearance of being made up of lamellæ, or plates. The test for starch is a solution of iodine. Iodine dissolved in absolute alcohol gives a brown color to dry starch. If any moisture is present, the color is blue. As an ordinary test for starch, iodine dissolved in a watery solution of potassium iodide may be used. If a little of this is run under the cover, the characteristic blue color of the iodide of starch appears.

The changes caused in the starch granule by the gradual addition of a strong solution of caustic potash are instructive. As the caustic potash is run under the cover-glass, the starch granule is seen to swell, and the hilum and laminations become more distinct. Soon the lamination becomes less distinct as the granule becomes more glassy and transparent. Dilute chromic acid is another reagent which renders the stratification more distinct.

Under polarized light the starch granules are brought distinctly into view, each with its characteristic black cross. A good specimen for the study of starch granules *in situ* is a section of a potato taken from just below the skin. A little of the juice of a potato, or the flour obtained by grinding to a powder a pea, bean, kernel of corn, wheat, or oats, also affords good material for study.

Cellulose. — Iodine imparts a yellowish brown color to cellulose, the fundamental material of the cell wall. If a little strong sulphuric acid is run into the specimen, the color is changed to blue. The best reagent for detecting cellulose, however, is Schultze's reagent or chlorzinc iodine. This is made by dissolving metallic zinc in hydrochloric acid, evaporating to the consistency of syrup with a piece of undissolved zinc remaining, and adding as much potassium iodide as will dissolve. Finally iodine is added until a deep reddish brown color is produced. This reagent colors cellulose violet.

Another useful reagent for the study of cellulose is ammoniacal cupric oxide. It is prepared by adding caustic soda or caustic potash to a strong solution of copper sulphate as long as a precipitate is formed. Filter, and wash the precipitate in water that has been boiled. Place the greenish mass in a bottle, and add just enough strong ammonia to dissolve it. When properly prepared, fibres of cellulose swell up and dissolve in it. To test the reagent, see if fibres of cotton are so acted on by it. It must be kept in the dark.

Lignified Cellular Tissue. — Aniline chloride dissolved in ten parts of alcohol and one part of hydrochloric acid imparts a yellow tinge to lignified cellular tissue. Aniline sulphate dissolved in ten parts of alcohol, or of water, and one part of sulphuric acid also gives a yellow color to lignified cellular tissue. Lignified cellular tissue turns yellowish green if treated with a solution of carbolic acid crystals in warm hydrochloric acid and subsequently exposed to the direct sunlight. A strong solution of chromic acid readily dissolves cell walls. In weaker solutions it causes the cell wall to swell. It is therefore a valuable reagent in studying cellular tissues. Schultze's reagent imparts a clear yellow color to lignified cellular tissue, and brings it into prominent view. If this reagent is allowed to act on the specimen for some hours, the result is more decided. Objects will keep in good condition in this solution for a long time.

Protoplasm. — Protoplasm is a viscid, transparent fluid often containing finely divided dark particles, occurring in the younger cells of plants. It is the living part of the plant. It is best studied in the hairs of nettles, the hairs of very young shoots of the pumpkin, squash, or cucumber, and the hairs upon the stamens of the cultivated spiderwort, *Tradescantia Virginica*. If some of these hairs are examined in a drop of water under *high* power, the moving currents of the protoplasm are visible. It is often necessary to warm the slide slightly to cause the movement to start. In studying the protoplasmic movements, remove the water, and run a little glycerine under the cover-glass in the usual manner, and observe the effects on the protoplasm. The action of alcohol may, in like manner, be observed. Strong alcohol kills the protoplasm, which is then much more readily acted upon by staining fluids. A nucleus, containing a nucleolus, may be seen with a high power, in the cells of successful preparations, suspended by threads, and carried about in the cell as the protoplasm is acted upon by the reagents. If tissues containing protoplasm are treated with a thick syrup of cane sugar, and afterwards with strong sulphuric acid, the protoplasm is slightly colored red. Protoplasm also absorbs iodine from solution, and becomes reddish brown.

After some experience has been acquired in the study of protoplasm in plant hairs, the various reactions for it may be applied to the young cellular tissue found at the growing points of stems, roots, and leaves. It is to be remembered that protoplasm can be studied best in young cells. As the cells increase in age, cavities, or vacuoles, are formed, and the protoplasm is pushed close up to the wall. If such a tissue be treated with a somewhat strong aqueous solution of common salt, the protoplasm is caused to contract and becomes more easily visible. In still older cells the protoplasm wholly disappears. In very young tissues the application of a stain — hæmatoxylin is especially good — often brings out a network of delicate fibres in the protoplasm.

Chromatophores. — The green color of plants is due to minute spherical or polygonal shaped particles imbedded between layers of protoplasm. When viewed under the microscope, they appear to be suspended between the cell wall and the vacuole, or sap cavity, in the central part of the cell. When treated with alcohol for some time, the color disappears, but the forms of the granules are still faintly visible as colorless bodies. The green pigment is called chlorophyll; the granules that bear the pigment are known as chlorophyll bodies. Other pigments give the characteristic colors to colored plants. Chromatophore is a general name for the bodies that bear the various colors. It is characteristic of chromatophores that starch granules are developed in them. It is not always easy to see these without special treatment. Cut a green leaf into several small pieces, and place the pieces in a drop of water under the microscope. The chlorophyll bodies are liberated, and from some of them the enclosed starch granules are set free. Exposure to direct sunlight for several hours previous to the examination will render success more certain. Treat with iodine solution, and the starch shows the characteristic blue color, while the uninjured chlorophyll bodies take on a brown color. The enclosed starch granules may also be demonstrated by treating material which has lain in alcohol for some time with caustic potash to cause the starch granules to swell, and afterwards treating with iodine solution to produce the blue color. It is to be noted that the origin of starch granules is in the chlorophyll bodies. As the chlorophyll bodies increase in age, they gradually become more and more transparent until they disappear, and the matured starch granules are left uncovered in the cells of the plant.

The prothallia of ferns, which are always found growing on the plant-pots in hot-houses where ferns are cultivated, are particularly favorable specimens in which to study chlorophyll. The Zygnemaceæ are also good material for study. Keep the specimens in the sunlight several hours, treat with iodine, and the masses of chlorophyll will be seen to be surrounded with starch granules.

Plant Crystals. — Deposits of crystalline earthy matters are of very common occurrence in the cells of plants. The crystals most commonly take the shape of a prism, an octahedron, or of needles. Some can be found in almost any plant. The cuticle of the onion shows fine examples of the prismatic and octahedral crystals of the oxalate of lime. Similar crystals may also be found in the bark of the rose around the bases of the prickles. Raphides, or needle-shaped crystals, of oxalate of lime are abundant in rhubarb, especially in the root, and in the epidermis of the bulbs of tulips and hyacinths. The petioles of begonia contain octahedral crystals of the same salt. Tubers of the dahlia which have been in alcohol for some days show somewhat spherical-shaped crystals of inulin. During the examination for inulin allow nitric acid slowly to replace the water.

Owing to the difficulty of isolating the crystals from the cellular tissues the chemical composition is not known in many cases. It is known, however, that crystals of the oxalate of lime are most common. The carbonate of lime is the second most common crystal found. These are easily distinguished by means of acetic and hydrochloric acid. If acetic acid is applied to the section, crystals of calcium oxalate are not affected; crystals of calcium carbonate dissolve with effervescence. If hydrochloric acid is applied to the section, crystals of calcium oxalate dissolve without effervescence; crystals of calcium carbonate dissolve with effervescence.

Polarized light affords a very delicate means of detecting the presence of crystalline or other earthy deposits in vegetable sections. Even if the crystals do not distinctly polarize, their forms, especially when quite transparent, are brought out in some positions of the Nicols.

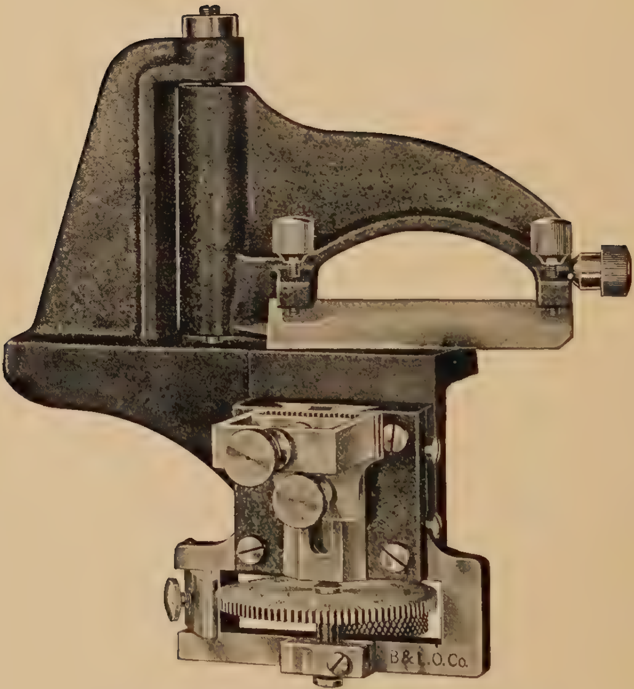
Animal Specimens.

Many of the directions already given for the study of vegetable specimens might be repeated for animal specimens. The methods described for the manipulation of the object under the microscope apply equally well to the study of both classes of objects.

Animalcules. — No attempt will be made here to describe the microscopic forms of animal life. The subject is too extensive for cursory treatment. Some good manual should be consulted for their identification. Any of these forms may be examined under the microscope if placed in a drop of water on the slide, and a cover-glass laid over the specimen. The cover-glass should be supported by bits of other thin glass. A shallow cell made as described in the following chapter makes a good live-box for the temporary examination of these specimens. The specimens are transferred by means of a piece of glass tubing used as a pipette.

The stagnant water of swamps furnishes countless varieties of microscopic animal life. The more quiet the water, the more abundant the animalcules. Seek out small, isolated pools. Collect some of the water around submerged objects. In collecting, it is best to place some of the aquatic plants in the collecting vessel with the water, disturbing the plants as little as possible. The animalcules cling in large numbers to the plants. A jar of this swamp water will furnish instructive entertainment for many an hour.

Water Sediment. — Allow a considerable amount of water to stand in a tall glass vessel for several hours. With a piece of rubber tubing, siphon off all but a small remnant of the water. With a pipette, place a very small drop of



Bausch and Lomb Clinical Microtome.
Fig. 38.

the sediment on a glass slip, and put a cover-glass over it. Examine with objectives of different powers, beginning with a low power. Solid particles of an earthy character will probably be found together with some microscopical forms of animal and plant life. The more impure the water, the more abundant these forms. The solid particles are probably either silica or the carbonate of lime. Run a little strong sulphuric acid under the cover-glass. Carbonate of lime is dissolved with effervescence of carbon dioxide, which will form numerous minute bubbles. Silica is unaffected by the sulphuric acid, and vegetable substances are more or less blackened.[1]

Striped Muscular Fibre. — The tissues of the larger animals may be prepared for examination by teasing them to pieces on the glass slip in a drop of water, glycerine, or some staining solution. The striated fibre of the voluntary muscles is thus easily prepared. Borax-carmine or hæmatoxylin is a good medium to employ. The fibrillæ, the nuclei, the transverse striations, and the sarcolemma, or investing membrane, are readily made out. The study should be begun with a low power, and continued with a high power.

A half per cent solution of common salt in water is also a good medium for this study. A preparation may be made by teasing up a bit of any of the active muscles of the legs of an animal in the salt solution. After the transverse striations have been studied, remove the salt solution and run acetic acid under the cover-glass. This causes the striations to show less distinctly, but the nuclei and the sarcolemma are brought more clearly into view. If now the acetic acid be washed out by applying successive drops of water to one side of the cover-glass and drawing them under by means of a piece of filter paper at the opposite side, and then a little borax-carmine stain be run in, the nuclei take a deep color and are rendered very conspicuous. They are somewhat elongated or fusiform, and seem to be at the very surface of the fibres.

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[1] The minute animal and vegetable organisms found in water supplies offer an extensive field for practical work. Whipple's *The Microscopy of Drinking Water* is a helpful book. John Wiley & Sons, New York.

Unstriped Muscular Fibre. — In a similar way a study may be made of the unstriped fibre of the involuntary muscles by teasing out a piece of the wall of the stomach or intestine. The elements of this muscle will be found to be elongated cells, each containing a nucleus situated near the centre, which stains more deeply than other portions.

Sections of Animal Tissues. — To obtain sections of soft animal tissues it is necessary to harden the specimen. This is most conveniently done by placing small pieces in alcohol for a few days. Better results are obtained if the specimen is gradually hardened by remaining a few hours in weak alcohol, say 10, 20, 40, 50, and 70 per cent and 95 per cent alcohol. The pieces should be not more than half an inch square and one-fourth of an inch thick, and a large volume of alcohol should be used. When sufficiently hard to give clean sections, the object may be held in the hand or between pieces of carrot or of pith moistened with alcohol, and sections cut by the freehand method already described. Or the object may be freed from alcohol by soaking in turpentine a few hours, and imbedded in paraffin by the method described in Chapter IX. Both unstained and stained sections should be examined. The staining is best accomplished by allowing the sections to lie in a watch glass with a few drops of the staining fluid until the right intensity of color is obtained. The best effects are secured when the specimen is treated, before and after staining, according to the directions given in the Appendix in connection with the formulas for the different staining solutions.

Blood. — Place on a cover-glass a small drop of fresh blood obtained by pricking a fold of the skin on the back of the wrist with a needle previously sterilized by passing it through the gas flame, and invert the cover-glass on a glass slip. Observe the numerous red corpuscles floating about in a colorless plasma. The corpuscles seem to attract one another, and are seen to move towards one another, forming rolls. The tendency to do this is increased if the cover-glass is slightly moved on the slip. The red corpuscles are seen to be round and very thin, regular in shape, disk-shaped, hollowing in the centre, or bi-concave. Among them the larger and irregular white corpuscles may possibly be distinguished, but it is not always easy to do this, as in health the white corpuscles are very few in number compared with the red corpuscles. In some diseases the relative proportion of the white corpuscles is much increased.

Fresh blood from a frog is more favorable for the study of the white corpuscles. The white corpuscles are relatively much more numerous in the blood of the frog than in human blood. The form of the white corpuscle will be seen to be very irregular, the appearance being very much like the amœba. If the slide is slightly warmed, a true amœboid movement may be seen in these corpuscles, as the irregular projections change in form and size, moving the corpuscle about. The structure of the corpuscle is granular in the central portion; clear and transparent in the outer portions. If the specimen is treated with acetic acid and then with borax-carmine, the nucleus will stain, showing one or more nucleoli; the outer portions remain unstained.

The red corpuscles of frog's blood are also very interesting. They are much larger than the corpuscles of human blood, oval in shape, and contain a distinct nucleus which is slightly convex, being thicker than the peripheral portions. If the specimen is treated with alcohol and then with hæmatoxylin or with borax-carmine, the nucleus takes a deep stain, the other portions remaining uncolored. Iodine is also a useful staining agent for blood corpuscles.

For further study make smears of blood and stain with Wright's or Hastings's modification of Leishman's blood stain. These stains are best bought ready prepared, as they are difficult of preparation. Place a minute drop of blood near one end of a glass slip. With the end of a second slip placed in close contact with the first and with a quick, sweeping motion of the hand, spread the blood in as thin and even a smear as possible along the entire length of the slip. Or, place the drop of blood on a cover-glass and lay a second cover-glass over it. Draw the cover-glasses apart, and a thin evenly distributed film of blood will be left on each. Allow the films to dry in the air. Cover with stain and allow them to stand about a minute. Then add water drop by drop until a delicate, irridescent, metallic lustre forms on the surface. Allow this to remain about three minutes. Wash somewhat thoroughly by holding the preparation in a large volume of water. Dry between filter papers.

These preparations may be examined unmounted with the oil immersion objective repeatedly for years, if the immersion oil is washed off each time with xylol, or they may be mounted permanently in balsam.

The double staining may also be accomplished by using successively different aniline stains; fuchsin and methylene blue, fuchsin and Bismarck brown, fuchsin and iodine green are recommended. In making the preparations, the cover-glass may be dried by passing it through the heated air several inches above a gas flame, holding the cover-glass with the forceps. Much care must be exercised, or the corpuscles will be shrivelled. It is better ordinarily to let the film dry at the ordinary temperature of the room.

The Circulation of the Blood in a Frog's Foot. — The interesting appearance of the blood circulating in the web of a living frog's foot may be seen at the cost of very little trouble. No special piece of apparatus is required, but a thin strip of wood five inches long and one and a half inches wide, with a hole three-fourths of an inch in diameter bored through it an inch from one end, makes an excellent frog plate. In the centre of a piece of cloth about eight inches square cut a small hole, and pass the frog's foot through it. Wrap the cloth around the frog and saturate it with water. With a soft string bind the frog loosely upon the strip of wood in such position that its foot comes over the opening. Tie threads to its toes, and so fasten them that a portion of the web is spread horizontally over the opening, and all is ready for examination. A little care must be exercised that the strings are not drawn too tight, or the circulation may be stopped. The frog does not appear to be hurt, and after the first fright is over usually lies very quietly.

Cartilage. — Cut as thin a section of cartilage as possible, and mount in a half per cent salt solution. Cartilage from the sternum of a frog gives particularly favorable specimens. Slowly run under the cover-glass some iodine solution. The cells of the cartilage will be brought into clear view as they take a deep brown stain from the iodine. The cells occur singly, or two, three, or four together. When there are three, one is larger than the other two. The growth by cell division is evident from this arrangement of the cells. Each cell contains a nucleus; some contain two nuclei. The nuclei sometimes contain several nucleoli. Between the cells is a lifeless matrix which does not take the stain.

Bone. — Bone may be decalcified by treating it for several hours with very dilute hydrochloric acid. As the hydrochloric acid destroys some of the soft tissues, better results will be obtained if chromic acid is used, beginning with a one-sixth per cent solution. After twelve hours pour off this solution, and replace it with a one-third per cent solution, and after another twelve hours with a one-half per cent solution. Do not use a stronger solution than this, but use a large volume and allow the specimen to remain until softened. Wash thoroughly in water to remove the acid.

Sections may now be easily cut with a razor. Wash the sections in water to make sure that the acid is removed, and stain in borax-carmine, which imparts an intense color. Avoid overstaining, removing excess, if necessary, with acid alcohol.

It is not difficult to prepare sections of bone by grinding. A suitable piece of dry bone, a cross-section, for instance, cut as thin as possible with a surgeon's saw, is ground smooth on one side by rubbing it over a fine-grained whetstone or file. During the grinding, press gently but firmly upon it with the fingers. Now place a large drop of Canada balsam on a piece of glass about an inch square, and hold the glass with the forceps, or better with a spring clothes-pin, over the gas flame until the volatile portions are driven off. Allow it to cool, but before it is quite ready to set, place the smooth side of the bone on it, and press it gently down until the balsam is cold. Using the glass as a holder, continue to grind the bone on the whetstone or file until it is thin enough. Wash the surface with alcohol, and examine the progress of the work from time to time under the microscope, placing a drop of water on the specimen and covering with a cover-glass. The section may be thus studied on the bit of glass, or it may be very easily made into a permanent mount. Immerse it in chloroform or benzole until the balsam dissolves and the specimen slips off from the piece of glass. Use no force to remove it, or it will be injured. Place a drop of balsam on a glass slip, and heat to drive off the volatile portions so that, upon cooling, the balsam becomes quite hard. Treat a cover-glass in the same way. Remove the section of bone from the chloroform or benzole, using a camel's-hair brush as a lifter, and drain off the fluid by touching the section with a piece of filter paper. Place the dried section on the centre of the slip, invert the cover-glass over it, and hold some distance above the flame until the balsam just melts. Set aside to harden. The object of this treatment is to prevent the balsam from penetrating the specimen and rendering the structure too transparent.

Epithelium. — Epithelium is to the internal cavities of the body what the skin is to the outside. It does not, however, form a firm and continuous covering to the underlying tissues. It exists in various forms of loosely joined cells. These forms run into one another insensibly, but are usually distinguished as pavement and columnar epithelium. The cells of the former are thin and flat. Their arrangement somewhat resembles the arrangement of the stones of a pavement. The cells of columnar epithelium are thickly crowded together, arranged like aggregates of columns. The columnar epithelium may have cilia or hairlike appendages.

To obtain specimens for examination, scrape with a dull knife-blade the inside of the cheek, the roof of the mouth, or the mucous membrane of the intestine of a recently killed animal, and mount the scrapings in water, or a half per cent salt solution. The cells are nucleated. Scrapings from the tongue or the roof of the mouth of a recently killed frog are especially good for the study of ciliated epithelium. The movements of the cilia are best studied by watching the specimen under a high power for some time, while the cells die, the movements of the cilia in full life being too rapid to be observed well.

The methods of studying vegetable and animal specimens may, of course, be indefinitely extended. If sufficient has been said to give the beginner a start in the right direction and awaken interest in these fascinating processes of investigation, the object of this chapter is attained. The use of so many different stains and reagents is wont to seem to the beginner an almost insurmountable obstacle to the work. The expense involved is very slight, and a little experience shows that the trouble of the manipulative processes is small. As nearly all the reagents and stains keep well, and some are even improved by age, they should be prepared in leisure hours and kept ready for use in suitable bottles. When the trouble of using is thus reduced to a minimum, there soon comes to be a fascination in studying the effects produced by different reagents and combinations of reagents. A previous knowledge of chemistry is, of course, an advantage in this work, but is by no means a prerequisite.

CHAPTER VI.

MOUNTING OBJECTS FOR THE MICROSCOPE.

THE process of mounting objects for the microscope for permanent preservation is very simple. Many different media are employed, but it is well for the beginner to become thoroughly familiar with the working of one before attempting to use others. One who has mastered the methods given in this chapter will find no difficulty in using any medium.

Mounting in Canada Balsam. — The medium most universally employed is Canada balsam. This medium presents the fewest difficulties. It is therefore well suited to the needs of the beginner. Canada balsam may be obtained of dealers in microscopical supplies in the pure form, or combined with various solvents, as benzole, xylol, or chloroform. These solutions of balsam, when properly prepared, harden much more quickly than the pure balsam. In their preparation the pure balsam is heated at a moderate temperature until the volatile portions are driven off, so that when cold the balsam is hard and chips easily. This hardened balsam is then dissolved in benzole, xylol, or chloroform. When an object is mounted in this solution, the solvent quickly volatilizes, and leaves the cover-glass securely cemented to the glass slip by the hard balsam. Ignorant or unscrupulous dealers sometimes simply mix fresh balsam with the solvent, and sell the mixture under the name of benzole, xylol, or chloroform balsam. Such a mixture is as slow in drying as the pure balsam.

If the object to be mounted contains moisture, as is ordinarily the case, it must first be thoroughly dehydrated, or a cloudiness will result around the object from the fact that the water does not mix with the balsam. The dehydration may, in most cases, be sufficiently accomplished by soaking for some minutes in strong commercial alcohol, commonly spoken of as 95 per cent alcohol. The use of absolute alcohol will insure perfect dehydration, but there are comparatively few cases where it is a necessity.

After dehydration the alcohol is removed, and the specimen cleared, by placing it for a few minutes in oil of cloves, turpentine, benzole, or some other substance which drives out the alcohol, and which is itself miscible with balsam. Perfect clearing is important, and it is often well to pass the specimen through two changes of the clearing agent. Both dehydration and clearing may be effected after the object is placed on the slide. The specimen is covered with a large drop of alcohol or of the clearing agent. After a few minutes this is drained off, and another portion of the fluid applied. Repeat the process still again for especial thoroughness.

A glass slip is thoroughly cleaned and warmed over a gas flame to drive off all adhering moisture. The slip is placed on the turn-table, the object is exactly centred, and a small drop of Canada balsam is dropped upon it. The cover-glass, thoroughly cleaned and warmed, is allowed to fall gently upon this. The cover-glass should be handled with the forceps to prevent leaving finger marks or stains upon it. One edge should be brought in contact with the balsam at one side of the object. It should then be slowly and gradually lowered to position to prevent displacement of the object by a too violent movement of the balsam. The cover-glass should then be pressed down and exactly centred. Gentle pressure may be applied, if desired, by means of spring clips; or by means of lead bullets slightly flattened by a blow from a hammer so that they will not roll; or by large-headed screws, which are excellent for this purpose.

The balsam may be allowed to harden at the ordinary temperature. If pure balsam is used, the hardening will require some weeks, so that it is often desirable to hasten the process. The slides may be placed in the oven of a water-bath, and a temperature of between 130° and 140° F. maintained for several hours. If a higher temperature is used, or if the heating is unduly prolonged, the balsam will turn yellow. Another way is to put the slides in the kitchen oven at night just after the fire is dumped. In the morning the balsam will ordinarily be well set. A steam radiator or the mantle-piece above a stove serves a similar purpose. The slides should of course be protected from dust. Each should bear a temporary label to prevent mistakes in identification.

This is all that is really necessary for a permanent mount. The object will be preserved indefinitely without further treatment. With practice it is not difficult to gauge the amount of balsam so that the space between the cover-glass and the glass slip will be exactly filled without overflow. If this has not been accomplished, and there is an overflow of balsam, the slide may be placed on the turn-table, and the thoroughly hardened balsam cut off with a chisel-shaped instrument. A little water placed on the slide will prevent the balsam chips from flying about, and will help in cleaning the slide. When the superfluous balsam has been dislodged by means of the chisel, the slide is placed under a gentle stream of water from a faucet and carefully rubbed with a small brush. The final portions may be removed with a piece of cloth wet with alcohol, after which the slide is carefully wiped with a dry cloth.

Mounting in Glycerine-Jelly. — To mount with glycerine-jelly requires rather more skill than to mount with Canada balsam, on account of the difficulty of getting rid of air bubbles, which must be removed before the mount is sealed. There are, however, no serious obstacles to be overcome. For many objects, noticeably delicate plant sections, glycerine-jelly is superior to balsam. Delicate tissues are shrivelled by dehydration. Glycerine-jelly does not require that the water should be removed from the specimen, as water and the jelly are readily miscible.

The bottle of glycerine-jelly is placed in a dish of water, and the water heated until the jelly melts. Carefully clean a slide, place it on the turn-table, put the object on its centre, and breathe upon it. Working quickly, place a drop of the melted jelly on the object, removing any air bubbles by pricking them with a needle. Breathe upon a clean cover-glass, put a little of the hot jelly upon it, invert it over the specimen, and centre exactly. If air bubbles are formed under the cover-glass, they may sometimes be removed by introducing a fine wire.

Another method of procedure is to place a small piece of the solid glycerine-jelly on the centre of the glass slip, and carefully warm until it melts. The object is placed in this, and the air bubbles are removed, if any are formed. A piece of the glycerine-jelly is placed on a cover-glass held in the forceps, and melted. The cover-glass is then inverted over the object and centred.

Set the slide away with a light weight upon it for twenty-four hours for the jelly to harden thoroughly. Remove the superfluous jelly with a knife and by washing in *cold* water, using a cloth wet with water to remove the final portions.

Glycerine-jelly mounts must be sealed with some tough cement to prevent the entrance of air and the parting of the cover-glass from the slide. Preliminary to doing this the glycerine-jelly around the edge of the cover-glass may be firmly set and rendered insoluble by slightly moistening it with a strong solution of potassium dichromate and exposing to the sunlight. The slide is placed upon the turn-table, and the potassium dichromate applied with a brush. When the slide is ready for the cement, a sable-hair brush with long hair is lightly charged with some transparent cement, such as shellac cement, or Berry's hard oil finish (see Appendix). The turn-table is rapidly whirled and a light ring of cement formed about the edge of the cover-glass. The brush is charged a second time, and the ring is reinforced by a second coat. The slide is now set aside for the cement to harden. Several additional coats should be applied at intervals until a firm ring has been formed to hold the cover-glass securely in place.

Mounting in Thin Fluids. — It is sometimes desirable to mount in clear glycerine, or in some fluid of even less consistency. The principal difficulty arises from the ease with which the cover-glass is displaced when it is attempted to apply the cement ring. To overcome this difficulty, after the cover-glass is in position over the object, and the superfluous fluid which runs out from under the cover has been removed with a piece of filter or blotting paper, place minute drops of melted paraffin or sealing wax at three equidistant points around the cover-glass. Upon hardening, these drops will hold the cover-glass in position while the rings of colorless cement are being applied. In many cases a shallow cell, such as is described in the next paragraph, is required to hold the fluid medium, especially when the object is of appreciable thickness.

Dry Mounting. — Some objects are rendered too transparent when mounted in any of the above-mentioned media. The markings on starches, scales of moths, diatoms, and many similar objects are much less distinct when imbedded in these media than when they are viewed dry. The principal objection to dry mounting is the fact that after some time moisture is liable to collect in the mount and obscure the view of the object. For dry mounts glass slips should be prepared with shallow cells, formed of rings of transparent cement, of a size to suit the cover-glass to be used. These cells should be well seasoned before they are used. Many of the slips may be prepared in leisure hours and kept on hand ready for use.

When ready to mount an object, run a *very thin* fresh coat of transparent cement on the outer edge of the hardened ring. Place the object in position on the slip, or, if light like starch or the scales of a moth, dust a little of it on a cover-glass. Lay the cover-glass, prepared side down, in position over the cement ring. The ring of fresh cement should be of such size that the edge of the cover-glass will extend into it slightly all around. Place a light weight on the cover-glass and set the slide away until the cement is hard. Then place it upon the turntable again and apply a *light* coat of cement over the juncture of the cover-glass with the cement ring. If too much cement is used, it will surely run under the cover-glass. When the first coat is dry, add another light one, and repeat until the mount is thoroughly sealed.

The sweating of dry mounts may in a measure be prevented, when the object will not be injured by exposure to the air, by making small openings through the hardened cement with a fine needle to give free circulation of the air through the cell. Much care must be exercised in making the opening, or the cover-glass will be broken.

Cells of paraffin are also much used for dry mounts. They are very easily prepared. The paraffin is heated quite hot and applied with a brush in the usual way of forming rings on the turn-table. The ring hardens in a few minutes and is then neatly trimmed down with a chisel-shaped instrument, using the turn-table as a lathe. A coat of transparent cement is applied to the ring, and the object is mounted and sealed as already described.

Mounting in Cells with Fluid Media. — It is often desired to preserve objects that are too thick to mount without some means of raising the cover-glass from the slide. Many delicate objects — whole insects, for example — would be crushed and much injured if mounted in the ordinary way, unless especially prepared for that style of mounting. All such objects may be mounted in cells of depths suited to each case. When the objects are quite thin, rings of transparent cement of sufficient thickness may be built up. These should be prepared beforehand and kept ready for use. A well-seasoned cell is much more reliable than one freshly made. In making these cells apply light coats of the cement at intervals, giving time for each to dry before the next application is made. For thicker objects, rings of glass, hard rubber, or metal should be purchased of some dealer in microscopical supplies. These are fastened to glass slips by smearing one side with transparent cement, placing them in exact central position on the slip on the turn-table, and setting aside with a light weight on them until the cement is hard. A coat of the cement is then run around the outside of the cell and allowed to harden.

In mounting, the cell is filled even full with Canada balsam, glycerine-jelly, glycerine, or other mounting medium to be used, taking care not to let the medium touch the upper surface of the ring. Place the slide on the turn-table and run a ring of transparent cement on the upper surface of the ring. Lower the cover-glass to position and see that there are no air bubbles. The edge of the cover-glass should be slightly imbedded in the ring of fresh cement. If skilfully done, the cement and atmospheric pressure will hold the cover-glass in position. Any overflow of the mounting medium should now be removed unless the medium is Canada balsam. It is generally better to wait for the balsam to harden before attempting to remove it. Aqueous media may be washed away, the cover-glass being held in position with spring clips, if necessary. After this cleaning, the cover-glass is secured in position by successive light rings of transparent cement.

It is important for success in cell-mounting that the upper surface of the cell be perfectly even and flat. Cement cells may be brought to this condition, when necessary, by means of a fine file, or by heating to soften the cement, and pressing squarely against the top of the cell with a glass slip. No attempt should be made to use curtain rings and other cheap substitutes for cells, as no amount of skill will enable one to succeed in closing the cell if its upper surface does not make perfect contact with the cover-glass all around.

Finishing Slides. — For practical purposes, slides made by the processes above described may be regarded as finished. The appearance and the durability of the slide will, however, be much improved by applying neat rings of colored cements. Before applying these colored cements, balsam as well as other mounts should be thoroughly sealed by the application of several light coats of transparent cement. When this cement has thoroughly hardened, the edges should be turned true with a chisel-shaped instrument, using the turn-table as a lathe. A somewhat broad ring of some colored cement may now be applied, entirely covering the transparent cement and extending slightly on to the cover-glass on the inner side and out on to the slip on the outer side. Give this cement time to harden and turn both edges true. A narrower ring of some contrasting color is now applied in such a manner as to leave a narrow ring of the first color on either side. When dry, a ring of a third color may similarly be applied so as to leave a ring of the second color on either side of it. With a little practice, rings of as many different colors as desired may be very quickly and neatly applied, and the appearance of the slide much improved.

There is in some quarters a professed contempt for this ornamentation of slides. Carried to excess it is unquestionably a serious waste of time. The contempt usually comes from those who have never acquired the skill to do a neat piece of work. One who has once acquired this skill knows that he possesses the ability to do neat work more rapidly than he could before do slovenly work. There is, too, a real pleasure in a piece of neatly executed mechanical work, and skill attained in this work is a training for the many other mechanical operations of the microscopist.

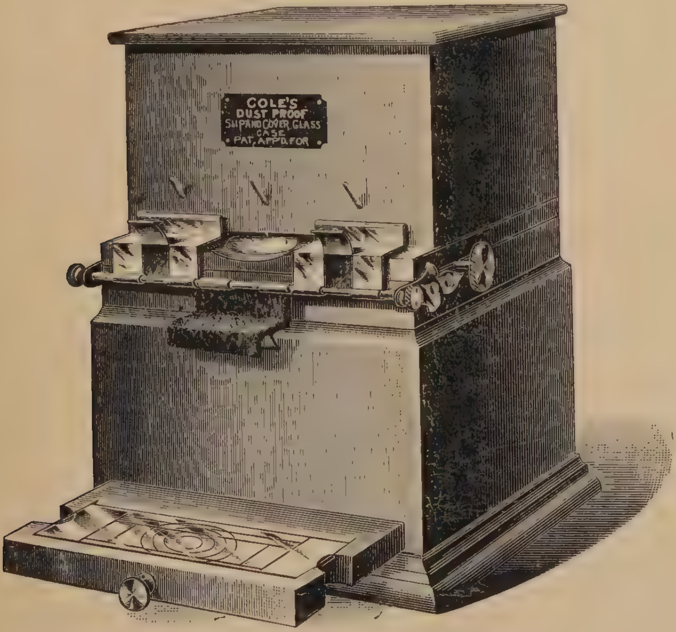
In applying cement rings, a long-haired sable brush should be used. The brush known among artists as the Rigger brush is excellent. The brush should first be lightly charged and the turn-table rapidly whirled. This first application forms a track which the cement will follow in the subsequent applications. The brush is now more heavily charged so that a *small* drop forms at the end. The turn-table is rapidly whirled, the brush held nearly vertically or slanted in a line tangent to the ring to be formed, and the drop of cement approached until it just touches the track formed for it. The judgment must be used as to the amount of cement to be now applied. As a rule it is better to apply the cement in light coats, giving time for each to harden. In this way the spreading of the cement is avoided.

It now remains to place neat permanent labels on the slide, and it is finished. These labels should state the common and scientific name of the object, call attention to any particular points of structure or preparation, name the mounting medium and stains employed, the thickness of the cover-glass, and any other facts which will be of value in future examinations of the slide.

Cleaning Glass Slips and Cover-Glasses. — It often happens even with experienced microscopists that imperfect mounts are made, and an easy way of cleaning the slip and cover-glass is desirable. A wide-mouthed bottle should be provided for this purpose, a pint fruit jar, for instance. In this place a strong solution of borax, washing soda, or caustic potash. Into this solution drop the spoiled slides, here to remain until a number have been so deposited, and until a convenient season presents itself to attend to them. The solution dissolves not only balsam, but all resinous media and cements. After a few days the cover readily slips off from even seasoned mounts, and the balsam and cements are easily removed by scraping with the thumb nail. Sharp instruments should not be employed to scrape a slide that is to be used again, as scratches are almost sure to result.

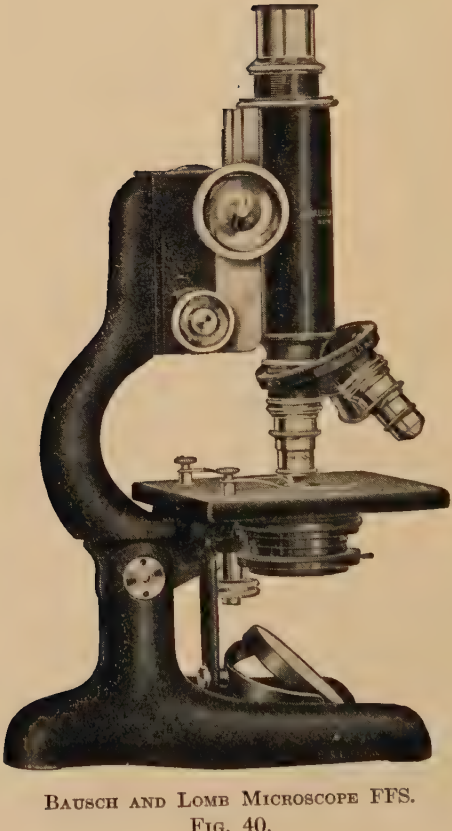
The writer uses ordinarily borax water, as being most agreeable to handle, and finds the glass clean enough for most practical purposes. To make sure that the slides and covers are optically clean, soak them for some days in the following mixture: Dissolve 10 grammes of potassium dichromate in 50 c.c. of water by the aid of heat; when the solution is cool, immerse the containing vessel in cold water, and pour into it 50 c.c. of sulphuric acid slowly and carefully with constant stirring.

Upon removing the slides and cover-glasses from this solution, wash them thoroughly in clean water. Fig. 39 shows a useful device for keeping them clean and free from dust.



Cole's Slide and Cover-Glass Case.

Fig. 39.



Bausch and Lomb Microscope FFS.
Fig. 40.

CHAPTER VII.

PRACTICE MOUNTING.

THE object of this chapter is to furnish illustrations of the methods of the preceding chapter, and to afford means of introducing the beginner to some of the manipulations necessary in the making of microscope slides. An object prepared for the microscope is not unfrequently an object of supreme mystery to one who is wholly inexperienced in the processes. Minute and explicit directions will be here given for a few simple, easily accessible, and easily prepared objects. If the beginner will follow these directions with patience and care, the mystery will be solved and be proved to be no mystery at all.

For this practice work the necessary equipment consists of a few glass slips and cover-glasses; a small bottle of benzole or xylol balsam; a bottle of sodium or calcium hypochlorite; a bottle of borax-carmine staining fluid; one each of iodine green, alcohol, turpentine, benzole, and a weak solution of caustic potash; a pair of improvised dissecting-needles; and plain forceps. With this equipment, not only the work of this chapter, but quite a wide range of investigation may be accomplished, and a considerable variety of work in the preparation and mounting of objects may be done. It is better, perhaps, to purchase the borax-carmine staining solution ready for use, though it is not difficult to prepare it according to the directions given in the Appendix. However it is obtained, it is of importance to know its alcoholic strength, and to soak the object to be stained in alcohol of the same strength before it is placed in the stain, and after staining to rinse off the the superfluous staining fluid in alcohol of the same strength. If these precautions are neglected, solid particles of carmine will be deposited on the specimen. The iodine green should be purchased in the solid form. A strong solution of this in alcohol should be made and filtered. This solution may be diluted with alcohol to any desired degree for use.

Insect Scales, Starch, and Similar Light Objects. — These objects afford interesting material for slides, and are especially easy of preparation. The wing of a moth is touched to a cover-glass, or a little starch is dusted on a cover-glass, and the cover-glass is inverted over a small drop of balsam on a glass slip. Hold the clean cover-glass in the forceps by one edge, bring the opposite edge into light contact with the balsam, and allow the cover to fall by its own weight gradually upon the balsam. Carefully press down and exactly centre the cover-glass. Pay no attention to air bubbles unless they are quite large, as they will disappear as the balsam hardens. The centring of the object and of the cover-glass may be accomplished on the turn-table, or by using the common expedient of drawing a figure one by three inches in dimensions on a slip of paper, finding its centre by drawing diagonals, and describing a circle the size of the cover-glass about this centre. The balsam is now hardened, and the slide cleaned and finished according to the directions given in the preceding chapter.

Hairs. — Hairs should be soaked in turpentine for some days to render them more transparent. They should then be cut in lengths suited to the size of cover-glass used, placed on a glass slip, a small drop of Canada balsam allowed to fall on them, and a clean cover-glass placed over them. Many hairs polarize finely. Among easily accessible and interesting hairs may be mentioned human hairs (especially those of the beard), caterpillar, rat, mouse, raccoon, and bat hairs, and the bristles of the hog. The hog's bristles are especially attractive objects under the polariscope.

Insects. — When the beginner has acquired a little experience in mounting the above-mentioned objects, he may advance to parts of insects, as the wing or foot of a fly, and to the mounting of small insects whole. Soak the object for twelve hours in a weak solution of caustic potash to cleanse it from any adhering dirt or oily particles, and to soften the harder portions of chitinous skeletons. If a whole insect is being treated, it should now be placed between two pieces of glass, and the soft visceral portions removed by gently and carefully pressing the pieces of glass together. The soft portions are then carefully brushed off, placing the piece of glass bearing the insect under water in a shallow dish and using two camel's-hair brushes, one to hold the insect, the other to remove the adhering matter. Renew the water until the caustic potash has been wholly washed out. Place the object in 95 per cent alcohol for twenty-four hours to remove the water. Then treat with turpentine. The longer the object remains in the turpentine, the clearer and more transparent will it become; but as the balsam has a similar effect, it may remain in the turpentine only long enough to become permeated. It is well to allow it to remain at least twenty-four hours. Warm a glass slip by passing it to and fro above a gas or lamp flame to drive off all moisture. Transfer the object to the centre of the slip and drain off the superfluous turpentine. Place a drop of Canada balsam on the object and cover with a previously warmed and cleaned cover-glass.

The insect world offers such variety that one might spend his life in studying and mounting insect preparations. A dissecting-microscope is of great use in separating and dissecting out by means of dissecting-needles the parts to be mounted. Parts which are too large to be mounted as above must, of course, be mounted in a cell prepared as described in the preceding chapter. The process of mounting is similar and but little more difficult than when no cell is used, as long as balsam is the medium employed.

Vegetable Sections. — Place some transverse sections of a plant stem, — a geranium stem is good for the first trial, — cut by the free-hand method described in Chapter V., in a considerable volume of sodium hypochlorite for several hours to bleach them. Wash thoroughly in several changes of water and transfer to 35 per cent alcohol. In a few minutes transfer to borax-carmine stain. Let the sections remain in the stain until upon the examination of one under the microscope it is found to be sufficiently colored. This operation will require a length of time varying from a few minutes to several hours, depending on the strength of the fluid. Transfer to slightly acid 35 per cent alcohol. In a few minutes transfer to a weak solution of iodine green in alcohol, where the sections should remain at least half an hour. They will probably now appear of a uniform green color. Place in 95 per cent alcohol until the green is removed from the parenchymal portions, but take care that they do not remain too long, or the green stain will be wholly discharged. Transfer to oil of cedar or to benzole to remove the alcohol and clear. After a few minutes mount in balsam.

Sections which will bear repeated transferals are best treated in watch glasses or individual butter dishes of white porcelain. When the sections must be left for some time in a fluid, two-drachm vials, to be obtained at the drug store, are very convenient, as they may be set away for hours tightly stoppered, and there is no loss by evaporation, as would be the case if watch glasses are used.

Preparations made by teasing, and any delicate tissues, may be passed through these processes on the glass slip. In each operation cover the specimen well with a large drop of the fluid. It is well to repeat the washing in alcohol and the clearing in oil of cedar or benzole to make sure of thorough work.

Animal Sections. — Place some sections of animal tissues, cut as described in Chapter V., in 70 per cent alcohol for a few minutes, transfer to Kleinenberg's hæmatoxylin, letting them remain until, upon the examination of one under the microscope, they are seen to be sufficiently colored. Remove to 70 per cent alcohol, and in a few minutes transfer to 95 per cent alcohol, then to absolute alcohol, and to oil of cloves. Mount in Canada balsam.

If it is desired to use other stains, follow the directions given in the Appendix in connection with the formula for each stain for the treatment before and after staining. Use a camel's-hair brush to handle the sections. Both animal and vegetable sections may be secured on the centre of the glass slip by means of white of egg or Schällibaum's collodion fixative, used as described in the Appendix.

Diatoms. — It is not the purpose here to go far into the very extensive subject of diatoms. The attention of the beginner is more particularly called to recent diatoms. At some pond, stream, or spring, gather some of the brown or yellowish brown slime adhering to any of the water plants. Place some of this slime in a test-tube with enough nitric acid to cover it, and boil for some minutes. Add a small quantity of potassium dichromate and boil again. The diatoms will usually be well cleaned by this process, if it is continued sufficiently long. Pour the contents of the tube into a large volume of water in a tall glass vessel and allow the vessel to stand undisturbed for several hours until the diatoms have settled. Without disturbing the diatoms at the bottom, decant off the liquid above them. Fill the vessel with clean water and allow it to stand again. Repeat this process until the acid has been entirely removed.

If, now, it is desired to separate diatoms of different densities, fill the vessel again with water, and let it stand until the heavier diatoms have settled. Decant off the water containing the lightest diatoms. Fill the original vessel again with water, and again let the heavier forms settle. Decant the lighter forms into another vessel. By a continuance of this process the diatoms of different weights are quite easily separated. The sediments from the different vessels used are finally collected in small bottles with a little water or alcohol.

To mount the diatoms, shake the bottle, place by means of a pipette a drop of the water containing the diatoms on a cover-glass, evaporate to dryness by holding the cover-glass above a flame, moisten with turpentine, place a small drop of balsam on the cover-glass, heat until the balsam boils, invert over a glass slip, centre, harden, and finish, as already described.

If one has the desire and patience to do the, as it seems to the writer, unprofitable work of arranging diatoms in figures, coat a cover-glass with gelatine fixative and let it dry. Under a low power arrange the diatoms in the desired pattern, using a mechanical finger, or simply a hog's bristle, or cat's whisker, attached to a wooden handle by means of wax. When the pattern is formed, or from time to time during its formation, breathe gently on the cover-glass to soften the gelatine. Upon drying, the diatoms will be held fast in the gelatine. Mount in balsam.

Some of the fossil diatoms may be cleaned by the process described above. Others it is more difficult to clean. Much of the foreign matter may in some cases be removed by heating the earth to a red heat, placing it for this process on a piece of platinum foil or of porcelain. It is frequently necessary to break down the mass of diatomaceous earth by boiling with a solution of caustic potash or caustic soda. These solutions attack the diatoms, forming silicates of potassium and sodium. If they are used, the action should not be allowed to continue long, but as portions of the mass are broken down, they should be poured into a large volume of water to wash out the reagent. It is, perhaps, better to break down the mass of earth with ammonia of moderate strength, as ammonia does not act upon the diatoms to any perceptible extent. When the mass has been broken down, pour the diatoms into a considerable volume of water, and allow them to settle. This requires several hours. Decant off the water, and place the residue in a dish in which it may be heated. If the foreign matter cannot now be removed by the use of nitric acid with potassium dichromate, use hydrochloric acid, aqua regia, and sulphuric acid successively.

Certain earths are best treated, after the breaking down with ammonia, by dropping in powdered potassium dichromate while the residue is boiling, and then pouring in strong sulphuric acid slowly and with constant stirring.

Some workers recommend boiling the diatoms in strong sulphuric acid, at the same time sifting in pulverized potassium chlorate. This process is dangerous, as violent explosions are liable to occur. It should be a last resort. Flocculent matter is often very troublesome. It may sometimes be removed by boiling the diatoms in a strong solution of soap. The processes are long and tedious. Fine sand can be removed only by the process of decantation already described for separating the diatoms of different weights. Gatherings of the thin coating of black mud on the bottoms of springs, ponds, and streams may be treated in this way and yield rich results in fresh diatoms.

CHAPTER VIII.

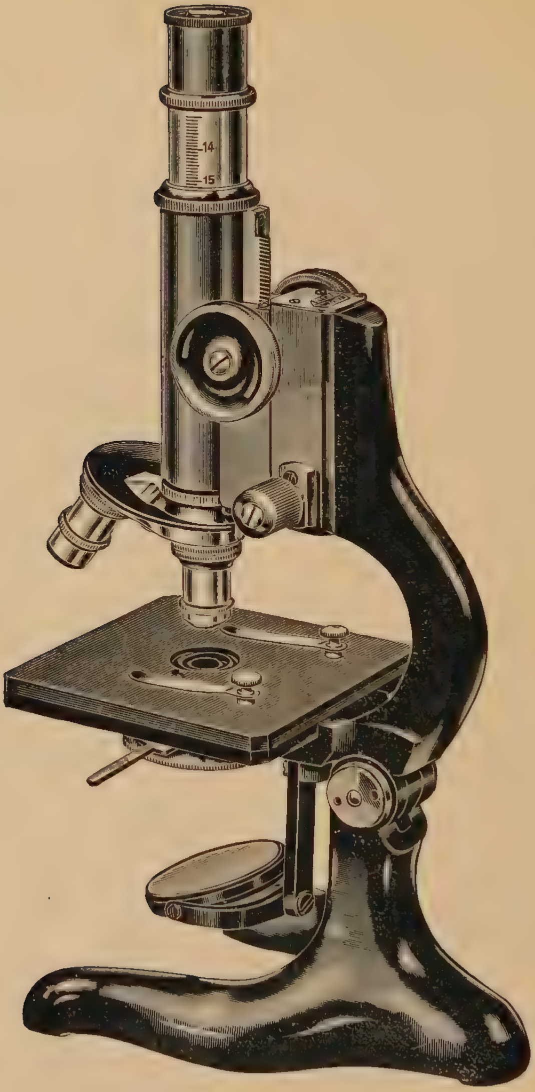
BOTANICAL SECTIONS.

In some of the preceding pages a few simple directions have been given for the preparation and mounting of vegetable specimens. If a considerable study of structural botany is to be made, more elaborate methods leading to more perfect results are desirable. Even if one is not especially a student of botany, the making of plant sections is attractive; from no other material can showy and interesting specimens be so easily prepared as from plant life.

For purposes of sectioning, vegetable specimens may be divided into two classes.

1. Hard specimens, or those that cannot be cut without softening.
2. Soft specimens, including annuals, young shoots, buds, seeds, cones, and similar products of plant life, many of which must be hardened or otherwise prepared by special processes.

Hard Specimens. — Most solid wood specimens may be sufficiently softened for cutting by soaking in water for some hours, changing the water occasionally, or by boiling in water for a few minutes. Three pieces of each specimen should be prepared to give sections in three different directions, — transverse, radial, and tangential. A transverse section is made across the stem in a direction transverse to the length. A radial section is made lengthwise the stem,



Leitz's Microscope LL.
Fig. 41.

along a radius of the circles of annual growth, or from the bark towards the centre of the tree. A tangential section is made lengthwise the stem, tangent to the circles of annual growth.

If no microtome is available, the pieces should be two or three inches long, that they may be firmly held in the hand, and about one-fourth inch in cross-section. Use a razor ground flat on one side, and follow the directions for cutting free-hand given in Chapter V.

For the microtome, specimens should be cut about one-fourth inch square and one inch long. If a microtome having a clamp is used, the softened piece of wood is fixed in the clamp and cut without further preparation. The nearer to the supporting clamp the section is cut, the more perfect it will be, as the springing of the specimen is reduced to the minimum. If a well-microtome is used, the specimen is fixed in the well by wedges of pine or other very soft wood which are sunk in the well, and do not come within one-fourth of an inch of the cutting plate. These wedges hold the specimen firmly, but leave one-fourth of an inch of it exposed for cutting. The specimen is placed at one side, close to the wall of the well, so that the wall may give it support and prevent the specimen from springing. In cutting, keep the knife and the specimen wet with water, dilute glycerine, or a mixture of glycerine and alcohol, using a camel's-hair brush to apply the liquid. Transfer the sections from the knife to a glass of water with a brush, or by dipping the blade of the knife in the water to float the section off.

Soft Specimens. — All specimens of a consistency suitable to be cut without special preparation may, of course, be cut free-hand, or may be placed directly in the clamp of the microtome and be cut at once. In many cases, however, immersion in alcohol for a few days is an advantage, when the changes resulting from the alcohol are of no account, as the alcohol removes all resinous substances, which stain and clog the knife. If the specimen is made hard and brittle by the alcohol, immerse it for a short time in a mixture of alcohol and glycerine, half and half, which will restore it to a soft condition.

Specimens which are too soft to be cut directly may be held between two pieces of cork, the specimen projecting about one-fourth of an inch above the cork. The whole should be clamped low down in the jaws of the microtome clamp to prevent springing. Do not cut through the cork, as it dulls the knife. Some specimens are advantageously held between pieces of pith, carrot, potato, or similar substance which will not injure the knife. Others are so soft that they must be imbedded in paraffin or celloidin. Some even require to be hardened before they are imbedded. The specimen is treated with one of the fixing fluids mentioned in the Appendix. It is then placed in 50 per cent, 70 per cent, and 90 per cent alcohol, remaining in each several hours. Omit such of the lower alcohols as the fixing agent permits. Soft tissues thus hardened may be kept indefinitely in 90 per cent alcohol. Specimens collected in July or August may be thus kept for winter use. They are very conveniently kept in two-drachm vials. The specimen should be completely covered with the alcohol, and each vial should be labelled. The more woody specimens, which become hard and brittle in the alcohol, must be soaked twenty-four hours in the mixture of alcohol and glycerine before they are cut. Specimens may also be preserved in formalin, which has recently come into use and is largely supplanting alcohol as a preservative medium.

Imbedding in Paraffin. — Three grades of paraffin may be obtained of dealers in microscopical supplies: hard, melting at 130° F.; medium, melting at 125° F.; soft, melting at 120° F. They may be mixed in any proportions necessary to give the right consistency for the temperature of the room in which the sectioning is to be done. Paraffin that will melt at about 125° F. is suitable in most cases. For paraffin moulds, obtain at the gas-fitter's pieces of the brass tubing used as the casing for gas fixtures. These pieces should be about one inch long and from one to two inches in diameter.

Prepare the object by dehydrating in alcohol of gradually increasing strength, up to absolute alcohol. Soak in chloroform twenty-four hours, then twenty-four hours in chloroform in which has been dissolved as much paraffin as will dissolve. As chloroform is somewhat expensive, the spirits or oil of turpentine is often substituted for it.

Melt the paraffin over a water-bath, but be sure that it is no warmer than is necessary to keep it fluid, or the specimens may be shrivelled. Pour on to a piece of paper enough of the melted paraffin to make a circle a little larger than the mould to be used. As the paraffin begins to stiffen fix the specimen upright in it, the end to be cut being down. Place a mould over the specimen, sinking it down into the circle of paraffin, and fill it with melted paraffin. Set aside, for the paraffin to become thoroughly hard. The hardening may be hastened, if so desired, by placing the whole in cold water as soon as the paraffin has stiffened sufficiently to bear this treatment. When the paraffin is hard, pass the mould two or three times through a gas flame, and the paraffin cast may then be easily pushed out. Trim off the opposite sides so that it may be more easily held in the jaws of the microtome, and cut away the paraffin to within an eighth of an inch of the object on the end to be cut, and it is ready for sectioning. The cast may be held directly in the clamp of the microtome, or it may be fastened to the roughened end of a cork by means of a little melted paraffin, and the cork held in the jaws of the clamp. If a well-microtome is used, the object may be imbedded in paraffin in the well. Imbedded specimens may, of course, be cut free-hand in the manner already described. Transfer the sections with a small brush to chloroform, turpentine, benzole, xylol, toluol, naphtha, benzin, oil of bergamot, oil of origanum, or oil of cloves, any one of which will dissolve the paraffin. Remove this solvent by transferring to two changes of alcohol before staining.

Imbedding in Celloidin. — Celloidin may be obtained of dealers in microscopical supplies. It is a form of nitro-cellulose. It dissolves in a mixture of equal parts of ether and absolute alcohol. Prepare two solutions, one quite thin; the other, thick. The object, which for this method should be not more than one-third of an inch in length, is thoroughly dehydrated in alcohol and ether and soaked in the thin solution of celloidin for twenty-four hours or longer. It is then transferred to the thick solution, where it is to remain some hours. It may then be imbedded as follows: Place a little of the thick solution of celloidin on a cork and let it dry for a few minutes. Add successive layers of celloidin, letting each dry, until a considerable mass is built up. Transfer the object from the thick solution of celloidin to this mass and build the celloidin up over it. Weight the cork and immerse the whole in 82 per cent alcohol, and in twenty-four to forty-eight hours the celloidin will be sufficiently hardened to cut. Strong alcohol dissolves the celloidin and must not be used. Objects imbedded in celloidin may be kept indefinitely in 82 per cent alcohol. In cutting, keep the object and the knife wet with 50 to 70 per cent alcohol. Plant sections should be cut to the thickness of one row of cells, which varies in different specimens from $\frac{1}{400}$ to $\frac{1}{1200}$ of an inch.

These somewhat complicated processes demand careful and painstaking work, but the quality of the results with delicate tissues amply repays the trouble. The simple processes already described of cutting the sections without imbedding the specimen, or after imbedding it in pith or carrot, are amply sufficient for a great number of objects that are to be cut, and will naturally be used, on account of their greater simplicity, when good sections can be obtained by them. There are, however, many delicate tissues that cannot be cut into thin sections unless their parts are supported by some substance that will infiltrate into the specimen. Resort is then had to the more troublesome methods. Another method highly recommended for this class of objects by many experienced workers is

Imbedding in Transparent Soap. — The soap mass is prepared as follows: Any good white soap is cut into thin slices and thoroughly dried by exposure to the sun. The soap is then crumbled to a fine powder and mixed with 90 per cent alcohol to form a stiff mass. Glycerine and 90 per cent alcohol are then added till the proportions by weight are: soap, 10 parts; glycerine, 22 parts; alcohol, 35 parts. Heat gently until transparent.

Place the object in 90 per cent alcohol, imbed in the soap liquefied by heat, cut sections dry or wet with alcohol, and remove the soap with warm water or alcohol.

Staining. — The staining of vegetable sections is a very simple matter. Many sections take the stain more readily if they are first bleached. Sections cut without imbedding, or after being freed from paraffin, are placed in water and then in an aqueous solution of calcium or sodium hypochlorite in a stoppered bottle. The time required for bleaching varies with different specimens from one or two hours to one or two days. The specimens must be watched to guard against maceration. If the action is slow, decant the calcium hypochlorite and add a fresh portion from time to time. The bleaching will be more complete if the sections are transferred from the calcium hypochlorite to very dilute hydrochloric acid for a few minutes. After the acid wash thoroughly in water to remove the acid. The sections may now be stained in carmine, hæmatoxylin, or in aniline dyes. The Appendix gives formulas for preparing the staining solutions, but the beginner is advised to obtain the stains ready prepared by some dealer in microscopical supplies. After some experience has been acquired with the ready-prepared stains, trials may be made in preparing one's own stains. It is best to confine one's self to a very few kinds until some experience has been gained. As a rule alcoholic stains are more satisfactory than the aqueous stains, if the object is to be mounted in balsam. In using the carmine stains it is to be remembered that it is very important that the object be immersed in alcohol of the same strength as the stain before staining, and transferred from the stain to alcohol of the same strength. If this precaution is not observed, solid particles of carmine will be precipitated in the cells of the tissue. The object is then passed through the higher grades of alcohol to complete dehydration.

Objects cut in celloidin may be transferred to water or dilute alcohol and stained in carmine or hæmatoxylin. The celloidin is but very slightly colored, and the sections are mounted without removing the celloidin. Aniline dyes stain the celloidin, and must not be used for these sections. After staining transfer to alcohol of the same strength as the stain, then to water, then to glycerine, in which the sections should soak some minutes before mounting. Mount in glycerine or glycerine-jelly. If Canada balsam is to be the mounting medium, transfer the section from the stain to alcohol of the same strength as the stain, dehydrate in 95 per cent alcohol very quickly, clear with chloroform, carbolic acid dissolved in turpentine, or oil of origanum, but *not* with oil of cloves, which dissolves celloidin.

It is to be remembered that the object of staining is to differentiate structure, and that that staining is most successful which brings out most perfectly the different parts of the specimen. Overstaining obscures structure and defeats the very purpose for which the staining is undertaken. Delicate staining also, as a rule, gives results that are in better taste than deeply stained objects. The thicker the section, the more careful must one be not to overstain. It is also to be remembered that many specimens are not suitable for double staining. The more homogeneous the structure, the less likely is the specimen to take two different stains.

The same dye prepared by different formulas acts differently on the same tissues. Experience alone can tell which stain, or which combinations of stains, will give the most satisfactory results. The same staining fluid also acts quite differently on sections of different plants. The acidity, alkalinity, or other chemical properties of the plant tissue modify the effect of the stains, so that each plant is, as it were, a law to itself in its behavior towards the staining fluid.

Order of Procedure with Vegetable Specimens to be cut in Paraffin.

1. *a.* If the material is fresh, harden in alcohol of gradually increasing strength up to absolute alcohol.
   b. If the material is alcoholic and hard, soak in alcohol and glycerine, equal parts, until soft; if the material is alcoholic and soft, omit this step.
2. *a.* Remove alcohol by soaking in benzole or oil of cloves. Soak 24 hours in benzole containing all the paraffin it will dissolve. Or,
   b. Soak in chloroform 24 hours, then 24 hours in chloroform in which has been dissolved as much paraffin as will dissolve.
3. Imbed in paraffin.
4. Cut sections. Fix to slide. See fixatives noted in Appendix.
5. Remove paraffin by soaking in chloroform, turpentine, benzole, or oil of cloves.
6. Remove chloroform, turpentine, benzole, or oil of cloves by soaking in two changes of 95 per cent alcohol. Bleach the section, if it seems desirable.
7. *a.* Place in 35 per cent alcohol.
   b. Stain in dilute Grenacher's Borax-Carmine.
   c. Place in 35 per cent alcohol, to which has been added a drop or two of hydrochloric acid.
   d. Pass through 50 per cent, 70 per cent, and 95 per cent alcohol.
8. *a.* Stain in an alcoholic solution of iodine green or methyl green, made very dilute.
   b. Wash out excess of stain in 95 per cent alcohol, and place for a few minutes in absolute alcohol.
9. Clear in oil of cedar or benzole.
10. Mount in pure Canada balsam.

Number 8 may be omitted. In this case, place the sections in absolute alcohol, clear in oil of cedar, oil of cloves, oil of marjoram, oil of turpentine, or benzole. Mount in pure balsam or in balsam dissolved in xylol, benzole, or chloroform. Sections stained in the aniline dyes are liable to fade in oil of cloves and in xylol, chloroform, or benzole balsam, or in glycerine, or glycerine-jelly. They should, therefore, be cleared in benzole or oil of cedar and mounted in pure balsam. Even then the aniline dyes are not very lasting for permanent mounts. Sections stained with these dyes should be kept in the dark, as exposure to strong light fades the colors.

If the sections are to be mounted in glycerine or glycerine-jelly, the above order may be adhered to through the staining. The sections pass from the stain into alcohol of the same strength as the stain, then into glycerine. They are soaked some time in glycerine, and mounted in glycerine or glycerine-jelly.

In the place of numbers 7 and 8 may be substituted any of the stains mentioned in the Appendix, taking care to treat the specimen before and after the staining according to the directions given with the formula for each stain.

Order of Procedure with Vegetable Specimens to be cut in Celloidin.

1. *a.* If the material is fresh, dehydrate in alcohol of gradually increasing strength.
   b. If the material is alcoholic and hard, soak in alcohol and glycerine, equal parts, until soft; if the material is alcoholic and soft, omit this step.
2. Soak in a mixture of equal parts of alcohol and ether for 24 hours.
3. Soak 24 hours in thin celloidin.
4. Soak 24 hours in thick celloidin.
5. Imbed in celloidin and immerse in 82 per cent alcohol for 24 to 48 hours.
6. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol. Keep the knife very oblique, and cut with a quick, steady stroke. If the sections will permit, remove the celloidin by soaking the sections in absolute alcohol; in this case, the treatment in staining and the subsequent processes may be the same as for specimens cut in paraffin. If the celloidin is not to be removed, which must be the case with all delicate sections, pass on to 7.

7. *a.* Place in 35 per cent alcohol with the celloidin on the sections.
   b. Stain in dilute Grenacher's Borax-Carmine.
   c. Place in 35 per cent alcohol to which a few drops of hydrochloric acid have been added.
   d. Place in 82 per cent alcohol.
8. Fix sections on slip with ether vapor or a small drop of ether applied to the sections, if the celloidin was allowed to remain on them.
9. *a.* Place in 95 per cent alcohol for not more than 30 seconds.
   b. Place on a glass slip and flood with carbolic acid dissolved in turpentine, or with chloroform, to clear the section. Apply the clearing agent until the celloidin is perfectly transparent.
   c. Working quickly, drop on benzole or chloroform balsam, and cover with a cover-glass.

The alcohol of commerce is often much below 95 per cent in strength. It may be necessary to use the so-called absolute alcohol for dehydrating if chloroform is used for clearing. Care must be taken not to allow the sections to remain in the alcohol long enough for the celloidin to dissolve.

In place of number 7 hæmatoxylin or one of the other carmine solutions may be substituted, remembering the precautions as to treatment before and after staining. To secure success with the celloidin method, the dehydration must be rapidly accomplished. The essential oils, such as oil of cloves, oil of cedar, etc., either dissolve the celloidin or cause the section to shrivel, and should not be used by preference, although it is true that they are so used by some workers.

Delicate Plant Tissues. — The methods already outlined are amply sufficient for the coarser plant structures and for work in which the preservation of cell-contents and the prevention of shrinkage in the tissues are not of great importance. When, however, one is dealing with delicate plant tissues, such, for instance, as root tips, terminal buds, prothallia of ferns, ovaries and spermaries, and similar young and tender growths, in which the preservation of the cell-contents and the prevention of shrinkage is of prime importance, the utmost care must be exercised in killing and fixing the contents of the cells, in removing the water by gradual dehydration, and in preventing sudden changes of temperature in preparing the object for imbedding in paraffin. At the risk of some repetition the methods of procedure requisite for the best results in delicate work will be detailed.

With these classes of tissues, killing the protoplasm and fixing the cell-contents as near as possible in their natural condition must first be accomplished, and upon the success of this step will depend the value of the future result. Killing and fixing the cell-contents may be done either by the use of fluids which operate so slowly that the tissues do not change form or by the use of fluids which act with great rapidity. Whatever fixing or hardening fluid is used, it is important that its volume be many times the volume of the object treated.

Dilute alcohol is much used in killing and fixing plant tissues. The delicate specimens are placed in 10 per cent alcohol, and then transferred to 20, 35, 50, 70, and 95 per cent and absolute alcohol. They are allowed to remain in the 10 per cent alcohol from half an hour to an hour, according to the size and character of the specimen. At each succeeding step the time is doubled. The specimens must not be allowed to remain too long in any of the alcohols below 70 per cent, as maceration is likely to ensue. They may remain indefinitely in the alcohols above 70 per cent. When the alcohol which corresponds with the alcoholic strength of the stain that is to be used (see Appendix for treatment before and after each stain) has been reached, the specimen may be stained in the mass, if desired. Otherwise the process is continued to perfect dehydration, and the sections, after cutting, are stained on the slide or in watch glasses.

The use of Schultze's dehydrating vessel saves much time in making the transfers, and also avoids the injury to delicate specimens which is almost sure to result from changing them from one vessel to another. A modified form of Schultze's vessel is shown in Fig. 42. Another modified form may be improvised as follows: Partly fill a large-necked bottle with absolute alcohol, placing anhydrous copper sulphate or calcium chloride in the bottom to keep the alcohol up to full strength. Select a test-tube of about the same length as the bottle. Cut the bottom off from this tube and prepare a false bottom or diaphragm of chamois skin. The piece of chamois skin should be cut circular in shape, of a diameter somewhat larger than that of the tube; it is held in the tube by a coil of three or four turns of spring brass wire. In putting this diaphragm in position, the chamois skin is laid over the coil of wire, which is then gently pushed

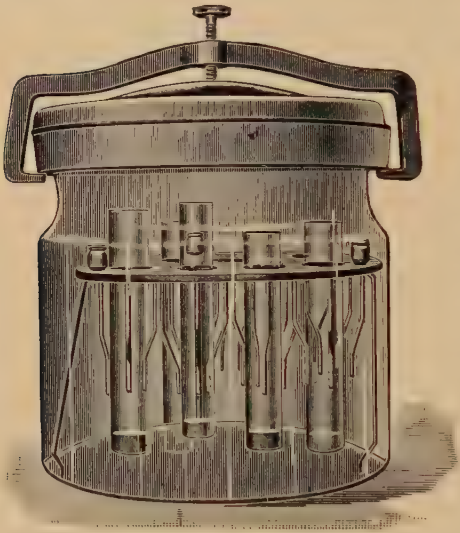


FIG. 42.

up into the tube so that the chamois skin comes between the coil and the inside of the tube all around. The tube thus prepared is to stand in the bottle; it should not rise so high in the neck that the bottle cannot be closely stoppered; it should also rest on the bottom of the bottle, or on supports placed on the bottom, in such a way that the absolute alcohol will have easy entrance to it. This tube is partly filled with 50 per cent alcohol. A second somewhat shorter tube, that will just rest in the first tube and be supported by its rim, is also fitted with a chamois skin diaphragm and is partially filled with 10 per cent alcohol. The delicate plant tissues are placed in the tube containing the 10 per cent alcohol; this tube is placed within the tube containing the 50 per cent alcohol; and this is placed in the bottle, taking care to have the liquids at the same level in the three vessels. Stopper the bottle tightly.

The operation is by osmosis. The dilute alcohol passes through the chamois skin diaphragm of the short tube into the 50 per cent alcohol, and the 50 per cent alcohol passes up into the dilute alcohol. At the same time the 50 per cent alcohol passes down into the absolute alcohol, the water of the weaker alcohol is absorbed by the copper sulphate or calcium chloride, and absolute alcohol passes upward. By this interchange of fluids the alcohol in the tube containing the specimens gradually becomes of full strength, and perfect dehydration of the specimens is accomplished in from twenty-four to forty-eight hours. The specimens may of course remain in the apparatus indefinitely, as the alcohol will preserve them perfectly. By this process the dehydration is so gradual that the cell-contents are killed and fixed in the cells without shrinking.

Picric acid is a valuable killing and fixing agent. It may be used in concentrated aqueous or alcoholic solution. Treat objects with this solution from two to twenty-four hours according to their character and size, remove them, and wash out the acid thoroughly in 95 per cent alcohol. This may take a number of days, but must be thoroughly done. Any alcoholic stain may be used.

Picro-sulphuric acid may be used in place of picric acid. Mayer's formula is: Water, 100 parts; sulphuric acid, 2 parts; picric acid, all that will dissolve. This may be used full strength or may be diluted with three times its volume of water. The object remains in the solution from three to twelve hours. It is then thoroughly washed in 70 per cent and the higher alcohols. Thorough washing is important, but this solution is more easily removed than pure picric acid. Warm alcohol will remove it much more rapidly than cold. Safranin in alcoholic solution is a good stain; so, too, are most alcoholic carmines and hæmatoxylins, if the acid has been thoroughly washed out.

Osmic acid may be used in the form of a solution or of a vapor. Aqueous solutions of osmic acid do not keep well. Lee therefore recommends that the osmic acid be dissolved in a 1 per cent aqueous solution of chromic acid, in which form it keeps well and is fully as efficient as when dissolved in water only. The solution should be quite weak, $\frac{1}{10}$ to 1 per cent. Small objects may be exposed to osmic acid vapors by placing them in a drop of water on a slide and inverting over the mouth of a bottle containing solid osmic acid or 1 per cent of osmic acid dissolved in 1 per cent aqueous chromic acid. Treatment with the vapor is always to be preferred when it is applicable. The time of treatment with either the solution or the vapor should be from one or two minutes to several hours, or even twenty-four hours, according to the size and character of the object. The acid should be washed out in water. Osmic acid blackens organic matter, but the color may be removed by subsequent treatment with a 4 or 5 per cent solution of commercial hydrogen peroxide in 70 per cent alcohol, or in a 1 per cent solution of chromic acid in water. The chromic acid should be washed out with water followed by alcohol. The objects should be transferred to the alcohol in the dark, as alcohol causes a precipitate with chromic acid if the two are brought together in the light. Treatment with Müller's, Erlicki's, or Merkel's solution from twelve to twenty-four hours also tends to remove the blackening. Stain with hæmatoxylin, picro-carmine, or ammonia carmine.

Merkel's solution is recommended as a fixing medium for very delicate objects. It consists of equal parts of a $\frac{1}{4}$ per cent aqueous solution of chromic acid and a $\frac{1}{4}$ per cent aqueous solution of platinum chloride. It should act from one to several days. Wash out in 35 per cent alcohol, stain in Grenacher's borax carmine, wash again in 35 per cent and the higher alcohols. Or after the Merkel's solution wash in 70 per cent alcohol, stain in Kleinenberg's hæmatoxylin, and complete the dehydration after staining. Objects treated with Merkel's solution or with chromic acid in any form stain with considerable difficulty. The acid must be thoroughly washed out; the staining fluid must act some time, and the sooner it is applied after the object has been freed from the chromic acid solution, the surer and better will be the results.

Corrosive sublimate (*mercuric chloride*) may be used in a saturated aqueous or alcoholic solution. Allow it to act from a few minutes to twenty-four hours according to the size and character of the object, and wash out in 70 per cent alcohol to which a little tincture of iodine or iodine dissolved in potassium iodide has been added. Follow with the higher alcohols, washing very thoroughly. The objects must not be touched with any implement of iron until the corrosive sublimate has been wholly washed out, as otherwise a precipitate of metallic mercury will be formed.

A solution of corrosive sublimate in 1 to 5 per cent aqueous acetic acid is also highly recommended as a fixing agent. Wash out in water or alcohol, preferably the latter. Objects fixed in corrosive sublimate should be imbedded and cut as soon as possible. Prolonged action of alcohol causes such objects to become brittle. Specimens treated with corrosive sublimate stain well, especially in the carmines, which may be used before the corrosive sublimate has been wholly removed, washing out in alcohol to complete dehydration.

Many other killing and fixing agents might be added to those already given, but this book does not aim to be a cyclopædia of methods. Enough of the standard solutions have been given to enable the beginner to obtain a knowledge of the general method of procedure. Other fixing and hardening agents are given in the following chapter and in the Appendix. The advanced worker must be referred to the extensive literature of this subject which is scattered in the pages of many scientific journals and monographs. Zimmermann's *Botanical Microtechnique* may be mentioned as a helpful book.

The general method of procedure with specimens that have been killed, fixed, and hardened by any of the above methods is as follows: They are transferred to absolute alcohol, cleared in chloroform, oil of cedar, xylol, or turpentine, and imbedded in paraffin; or they may be imbedded in celloidin. It is equally important in delicate work that no sudden transferals be made in these steps. If the specimens are to be imbedded in paraffin, place them with a small quantity of absolute alcohol in a test-tube and, by means of a pipette, introduce a clearing agent (chloroform, oil of cedar, xylol, or turpentine) very carefully into the bottom of the tube. Allow the tube to stand undisturbed, and the specimens will settle into the clearing agent. Now decant the mixed fluids and pour in some of the clearing agent undiluted. Let this stand until the objects are seen to be cleared. Then add thin shavings of paraffin until no more will dissolve. Place the specimens in this mixture on a water-bath and very gradually raise the temperature to 100° F., adding all the paraffin that will dissolve. Gradually pour off this mixture of clearing agent and paraffin, and add paraffin until the objects are immersed in pure paraffin, raising the temperature very gradually to 120° or 125° F., or the melting point of the paraffin, taking care that the temperature does not rise higher than is absolutely necessary to melt the paraffin. Imbed in paraffin in the usual way. Cut the sections. The sections may be fixed to the slide with Mayer's albumen or Schällibaum's collodion fixative, or they may be treated in watch glasses. Remove the paraffin with chloroform, xylol, or turpentine. If the specimens were stained in the mass, mount at once in balsam. If the specimens were not stained in the mass, treat with alcohols of gradually decreasing strength until the alcoholic strength of the stain to be used has been reached. After staining, dehydrate by successive treatments with alcohol of gradually increasing strengths to perfect dehydration, clear in oil of cedar, oil of cloves, xylol, benzole, or chloroform, and mount in balsam.

If it is desired to mount in glycerine or glycerine-jelly, place the objects, after they have been freed from paraffin and dehydrated, in a mixture of equal parts of alcohol and glycerine. Let the alcohol evaporate, transfer the objects to fresh glycerine, and mount in glycerine or glycerine-jelly.

If the specimens are to be imbedded in celloidin, place them after the gradual dehydration in a test-tube with a mixture of absolute alcohol and sulphuric ether, and introduce by means of a pipette some thin celloidin into the bottom of the tube. After the specimens settle into the celloidin, pour off the mixed solution and add thin celloidin. Let them remain in this twenty-four hours, decant the thin celloidin, and substitute thick celloidin. After twenty-four hours imbed in celloidin in the usual way. Cut the sections. The sections may be fixed to the slide by blowing or pouring ether vapor upon them, or by placing a minute drop of ether on them. If the specimens were stained in the mass, dehydrate, clear in chloroform, and mount in chloroform balsam. If the specimens were not stained in the mass, apply alcohol of gradually decreasing strength until the alcoholic strength of the stain to be used is reached. After staining, dehydrate by successive applications of alcohol of gradually increasing strength to perfect dehydration. Clear in chloroform until the celloidin is perfectly transparent. Mount in chloroform balsam.

Another modification of the celloidin process meets with much favor with some. It is known as the collodion process. Celloidin is a form of collodion patented and manufactured in Germany, and 2 and 5 per cent solutions of it may be substituted for the collodion solutions in this process. Ordinary collodion is, however, more transparent. Aside from this there is, perhaps, little to choose between celloidin and collodion. After the specimens have been gradually dehydrated in alcohol, they are placed in a test-tube with a mixture of absolute alcohol and sulphuric ether, and some 2 per cent collodion is introduced into the bottom of the tube by means of a pipette. The 2 per cent collodion is prepared by dissolving 2 grammes of commercial gun-cotton in 100 c.c. of a mixture of equal parts of 95 per cent alcohol and sulphuric ether. After the specimens settle into the collodion, pour off the mixture and pour on pure 2 per cent collodion, in which the specimens are to remain from twelve to twenty-four hours. The 2 per cent collodion is then decanted, and 5 per cent collodion is substituted. This is prepared by dissolving 5 grammes of gun-cotton in 100 c.c. of a mixture of equal parts of 95 per cent alcohol and sulphuric ether. The specimens remain in the 5 per cent collodion from twelve to twenty-four hours. They may then be imbedded in collodion on corks, or better on short cylinders of wood, in the manner already detailed for imbedding in celloidin. Immerse the cylinders bearing the imbedded specimens in 82 per cent alcohol for twenty-four hours, or, if the objects are small, in chloroform for two or three days.

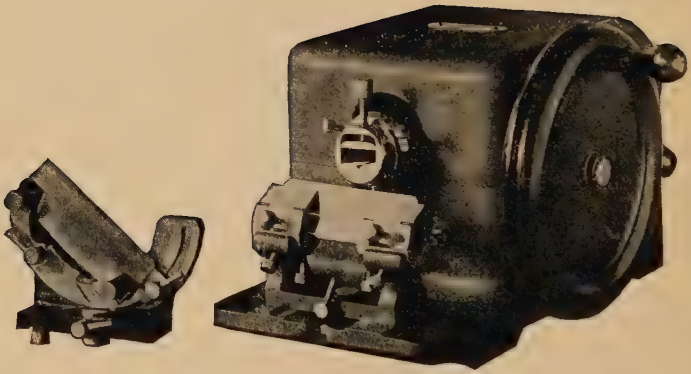
Or the following method may be pursued. Have ready pieces one inch long, cut from a cylindrical rod of wood. Wrap paper around one end of each little cylinder and allow it to rise half an inch above the end, thus forming a cylindrical box one half inch deep and one half inch in diameter. Fasten the paper securely with a pin or tack.

Wet the inside of the box with absolute alcohol, and pour in just enough thin celloidin to cover the top of the wood cylinder. Allow this to dry, and it will securely seal the bottom of the box and prevent bubbles of air from rising through the wood. Arrange the objects in position in the box, and pour in enough 5 per cent collodion just to cover them. Allow to stand, protected by a bell-glass, until the object becomes slightly exposed. Then add enough 5 per cent collodion again just to cover the object. Repeat as many times as may be necessary to have the object wholly covered as the collodion sets. Success with this method depends on the addition of the collodion in small quantities. If large quantities are added at once, air bubbles are almost sure to form. When the collodion has well set, immerse the cylinders in 82 per cent alcohol or in chloroform. In from one to three days the collodion will be well hardened. Strip off the paper, trim the top of the block square, and, holding the block in the jaws of the microtome, cut sections, keeping the collodion block and the knife wet with 82 per cent alcohol. The sections may be secured to the slide by blowing ether vapor on them or by applying a very small drop of ether and allowing it to evaporate. Clear in chloroform and mount in chloroform balsam, if the specimens were stained in the mass. Specimens that were not stained in the mass may be stained on the slide or in watch glasses with Grenacher's borax-carmine or Mayer's cochineal.

Celloidin and collodion sections may also be mounted in glycerine or glycerine-jelly. Transfer the sections as soon as they are cut to a mixture of alcohol and glycerine. Let the alcohol evaporate, transfer to pure glycerine, and mount.

Serial Sections. — It is frequently desirable to preserve a series of sections in the order in which they were taken from the object. Serial section cutting is more fully discussed in the next chapter. The processes there described are applicable to botanical as well as to animal sections. It is sufficient to state here that the sections are removed from the knife as fast as they are cut, or they are cut in chains or ribbons, arranged in a definite order, and fixed on a slide.

Freezing Processes. — Freezing processes are also discussed in the next chapter. For the purpose of obtaining many sections quickly for class study, these processes are valuable. It is also true that for sections to be studied by the methods of investigating fresh tissues, these processes cause less change in cell-contents than other methods of obtaining sections of vegetable tissues. These processes are not, however, adapted to securing sections of delicate tissues in their best condition for permanent preservation.



Spencer Rotary Microtome.

Fig. 43.

CHAPTER IX.

SECTIONS OF ANIMAL TISSUES.

Hardening. — Most animal tissues require hardening before they can be cut into thin sections. As a rule ordinary alcohol is the best as well as the most convenient reagent to accomplish this. With delicate tissues, however, much care is required, or they will be distorted and contracted because of the shrinkage which results from the fact that the alcohol removes the water from the tissue faster than the alcohol can take its place. It is usually better to treat the specimens with one of the fixing agents mentioned in the latter part of this chapter and in the Appendix, following the directions given in those places. After washing out the fixing agent thoroughly, pass the specimens through 50 per cent, 60 per cent, 70 per cent, and 95 per cent alcohol. The time should be short in the lower grades, as there is danger of maceration if the object is left too long in them.

The specimen to be hardened should be cut into small pieces. Persons not accustomed to the process ordinarily use pieces that are far too large. A piece one-third of an inch square and one-fourth of an inch thick will give more sections than can be used, and it is only a waste of time and of alcohol to use larger pieces. The latter not only harden less quickly, but less uniformly. Using a piece of one of the softer tissues — a piece of liver, for instance — of the above dimensions, treat it with one of the fixing agents, wash with water or alcohol as indicated, and place it in 50 per cent alcohol, two hours; 60 per cent, six hours; 70 per cent, twenty-four hours; 95 per cent, twenty-four hours; omit alcohols which the fixing agent permits. The volume of the alcohol should in each case exceed the volume of the specimen many times. Pint bottles with wide mouths are convenient vessels to contain the alcohol. Only one or two pieces of the tissue should be immersed at a time in each of these bottles, and the alcohol should be changed after having been used a few times, the weakest being thrown away and that in each of the other bottles being considered as of the next lower grade, the strongest alone being renewed.

Staining. — Many animal tissues in pieces of the size above mentioned may be stained *in toto* and better results obtained than when the staining is done after the sections are cut. Tissues which are found by experience not to stain well as a whole may be prepared for imbedding and cutting, and be stained after the sections are cut. Carmine and hæmatoxylin stains are most commonly used for animal preparations. The pieces are immersed for twenty-four hours in alcohol of the same strength as the stain to be used. If Kleinenberg's hæmatoxylin is to be used, immerse the pieces in 70 per cent alcohol; if Grenacher's borax-carmine is to be used, immerse in 35 per cent alcohol. It is impossible to give definite directions as to the time required for staining. The pieces must be watched, and when they appear to be deeply stained they are removed to alcohol of the same grade as the stain, made slightly acid with hydrochloric acid. They remain in the acid alcohol twenty-four hours, and are transferred to alcohol of the same strength, and pass through the higher grades to complete dehydration in absolute alcohol.

Imbedding in Paraffin. — The alcohol must now be removed from the specimen to prepare it for the penetration of the paraffin. Place in chloroform for twenty-four hours, adding a little sulphuric ether or oil of cloves to the chloroform; then place for another twenty-four hours in a solution of paraffin in chloroform, at the ordinary temperature; then in some of the same solution on the water-bath, maintaining a temperature of about 130° F. for several hours, or until bubbles cease to rise from the specimen; then in clear paraffin on the water-bath for several hours, at the same temperature. It is then ready for imbedding in the manner described under botanical specimens.

On account of the cost of chloroform, turpentine is most commonly used to remove the alcohol and prepare the specimen for the paraffin, though it is open to the objection that it has a tendency to render objects brittle. The above process need be modified only by substituting the turpentine for the chloroform. The process is, however, sometimes abridged. The object is placed in turpentine, at the ordinary temperature, for some hours, or until it appears clear. It is then placed directly in melted paraffin on the water-bath for several hours, and then imbedded. The temperature of the paraffin should not in any case be allowed to rise much above the melting-point of the paraffin.

Oil of cedar may also be used in connection with turpentine. It drives out the alcohol more thoroughly, and tends to better results. The object is transferred from alcohol to a mixture of alcohol and oil of cedar, half and half, then to clear oil of cedar, then to turpentine, or directly to melted paraffin on the water-bath.

If the casts are spongy, it is an indication of a lack of thoroughness in the processes. Such casts should be thrown back into the melted paraffin for further treatment. Casts made by the chloroform method will surely be spongy if the chloroform has not been wholly expelled by heating on the water-bath. When the details of this method are carefully attended to, the results cannot be surpassed.

Imbedding in Celloidin. — The method of imbedding animal specimens in celloidin is precisely the same as that detailed under botanical sections, and need not be repeated here. Perfect dehydration before immersing the object in the solution of celloidin is of the greatest importance. The celloidin method gives very perfect results.

Cutting Sections. — The cork bearing the celloidin cast is held in the jaws of the microtome clamp, and the surface of the specimen and the knife are kept wet with 50 to 70 per cent alcohol.

The paraffin cast is held directly in the jaws of the microtome, or is fastened to the roughened surface of a cork by means of melted paraffin. It is an advantage of this method that the sections may be cut dry. A section-smoother should be placed on the knife to prevent the sections curling. If there is a tendency to crumbling, smear the surface of the specimen with collodion before each section is cut and let it dry a minute or two.

Mounting. — To mount a section from a celloidin cast, transfer it to a glass slip, cover it with chloroform, drain, and cover again with chloroform, add pure or chloroform balsam before the chloroform evaporates, and cover with a cover-glass. To mount a section from a paraffin cast, dry a clean slide, place it on the turn-table, and with a camel's-hair brush apply to its centre a very thin coat of collodion fixative, transfer a section from the microtome knife to the centre of the slide with a camel's-hair brush, and smooth it out by pressing gently on it with a piece of smooth oiled paper. Place the slide in the oven of the water-bath, the temperature being maintained at about 130° F., to evaporate the clove oil from the fixative. The section will then be firmly secured to the centre of the glass slip. Remove the paraffin from the section by pouring upon it a large drop of turpentine. In a few minutes drain off the turpentine and add a fresh drop. It may be necessary to repeat this operation three or four times to remove all traces of the paraffin. Another way is to immerse the slip bearing the section in a bottle of turpentine for fifteen or twenty minutes. Wipe the slip free of turpentine and mount at once in Canada balsam.

If it is desired to mount in glycerine or glycerine-jelly, remove the turpentine by immersing the slip in two changes of 95 per cent alcohol, remove the alcohol by immersing in water, pour a large drop of glycerine on the object, drain it off, and then mount in glycerine or glycerine-jelly.

Order of Procedure with Animal Tissues when the Object is to be stained in toto *and cut in Paraffin.*

1. Harden in alcohol of gradually increasing strength.
2. *a.* Place in 70 per cent alcohol.
   b. Stain in Kleinenberg's hæmatoxylin.
   c. Place in 70 per cent alcohol, slightly acidulated with hydrochloric acid, if overstained; otherwise omit this step.
   d. Wash thoroughly in 70 per cent alcohol, and dehydrate with 95 per cent and absolute alcohol.
3. Soak in chloroform or turpentine for 24 hours.
4. Soak for 24 hours in chloroform or turpentine containing all the paraffin that will dissolve. Then gradually heat mixture.
5. Let stand in this solution on the water-bath, temperature about 130° F., for several hours.

6. Place in clear paraffin on the water-bath at the same temperature for several hours.
7. Imbed in paraffin.
8. Cut sections dry.
9. Fix on glass slip with collodion fixative.
10. Place the glass slip in the oven of the water-bath, temperature about 130° F., until the clove oil is evaporated from the fixative.
11. Remove paraffin with turpentine.
12. Mount in Canada balsam. Or remove the turpentine with alcohol, place in water, then in glycerine, and mount in glycerine or glycerine-jelly.

In place of number 2 may be substituted Grenacher's hæmatoxylin or any of the carmine stains, observing the precautions as to treatment before and after staining.

Order of Procedure with Animal Tissues to be cut in Paraffin and stained after they are cut.

1. Harden in alcohol of gradually increasing strength.
2. Clear by soaking in chloroform or turpentine 24 hours.
3. Place for 24 hours in chloroform or turpentine containing all the paraffin that will dissolve.
4. Place for several hours in melted paraffin on the water-bath, temperature about 130° F.
5. Imbed in paraffin.
6. Cut sections dry.
7. Fix sections on glass slip with albumen or collodion fixative.
8. Place slip in the oven of the water-bath, temperature about 130° F., to evaporate the clove oil from the fixative.
9. Immerse the glass slip bearing the section in a bottle of turpentine for 15 or 20 minutes.
10. Remove the turpentine from the section by immersing the slip in two different bottles of 95 per cent alcohol.
11. *a.* Cover the specimen with 70 per cent alcohol.
    b. Immerse the slip in a bottle of Kleinenberg's hæmatoxylin, or put a drop of the stain on the specimen, letting it remain until the section is sufficiently colored.

c. Immerse in 70 per cent alcohol, slightly acidulated with hydrochloric acid, if overstained; otherwise omit.

d. Dehydrate in 70 per cent, 95 per cent, and absolute alcohol.

12. Remove the alcohol with benzole or turpentine (do not use oil of cloves, as it will dissolve the collodion fixative), and mount in Canada balsam. Or, omitting the dehydration in number 11, place in water, then in glycerine, and mount in glycerine or glycerine-jelly.

Order of Procedure with Animal Tissues to be stained in toto *and then imbedded in Celloidin.*

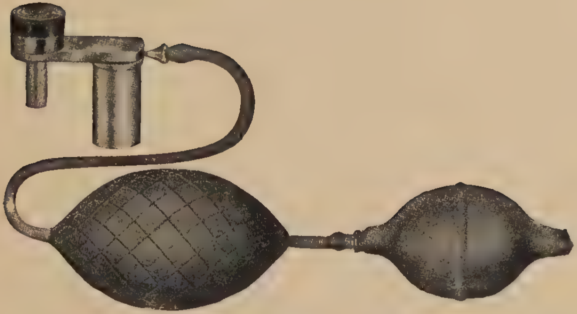
1. Harden in alcohol of gradually increasing strength.
2. Stain in hæmatoxylin or carmine, with the proper treatment before and after staining.
3. Dehydrate in 95 per cent and absolute alcohol.
4. Soak in alcohol and ether, equal parts, for 24 hours.
5. Soak in thin celloidin for 24 hours.
6. Soak in thick celloidin for 24 hours.
7. Imbed in thick celloidin on a cork in the manner already described.
8. Immerse in 82 per cent alcohol for 24 to 48 hours.
9. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol. Keep the knife oblique.
10. Place a section on the centre of a glass slip and cover it with 95 per cent alcohol. Immediately drain off the alcohol and cover with chloroform. Repeat until the section is clear. Or clear in carbolic acid dissolved in turpentine. Thorough clearing is very important. Mount in balsam.

Order of Procedure with Animal Tissues to be imbedded in Celloidin, the Sections to be stained after cutting and fixed in Position on the Glass Slip.

1. Harden in alcohol of gradually increasing strength to perfect dehydration in absolute alcohol.
2. Soak in alcohol and ether, equal parts, for 24 hours.
3. Soak in thin celloidin for 24 hours.
4. Soak in thick celloidin for 24 hours.

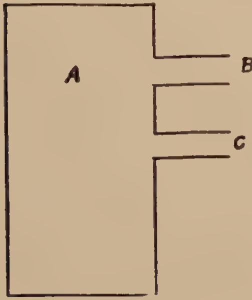
5. Imbed in celloidin.
6. Immerse in 82 per cent alcohol for 24 to 48 hours.
7. Cut sections, keeping the knife and specimen wet with 50 to 70 per cent alcohol. Keep the knife oblique.
8. Stain in hæmatoxylin or carmine.
9. Place in acidulated 95 per cent alcohol for 30 seconds.
10. Place on the centre of a glass slip, and fix in position by placing on the section just enough of a thin solution of bleached shellac in alcohol to cover it.
11. Place in the oven of a water-bath, temperature about 130° F., to evaporate the alcohol.
12. Clear in chloroform and mount in Canada balsam.

Freezing Processes. — The freezing processes of section cutting afford means of obtaining sections in abundance at the expense of very little labor and time. For simplicity of operation and for the quickness of the processes, the freezing microtomes have much to commend them. It is not possible, however, to secure very perfect sections of the more delicate tissues by these processes, on account of the distorting effects of the freezing and thawing. The processes are best adapted to cutting sections of fresh tissues, both animal and vegetable, for class use, when the utmost delicacy is not important. They are almost indispensable in laboratories where sections for study must be furnished to large classes. These processes are also well adapted for use by physicians who desire to make rapid examinations of fresh tissues. The freezing may be accomplished by means of a spray of ether or rhigolene, or by means of carbonic acid gas. For the use of ether or rhigolene an apparatus similar to that shown in Fig. 44 may be employed in connection with the microtome shown in Fig. 27, though it may be adapted to almost any form of microtome. The process with carbonic acid gas is less troublesome.



Ether Spraying Attachment for Microtome.

Fig. 44.



Attachment for Ice and Salt Freezing Mixture.

Fig. 45.

The apparatus for freezing with carbonic acid gas is shown in Fig. 46. *A* is a cylinder containing carbonic acid gas under pressure. *B* is the microtome shown in Fig. 27, fitted with a cylinder containing a spraying nozzle. The object, previously treated with mucilage, is placed on the top plate of the cylinder. The carbonic acid gas is allowed to flow, and the object is quickly frozen hard.

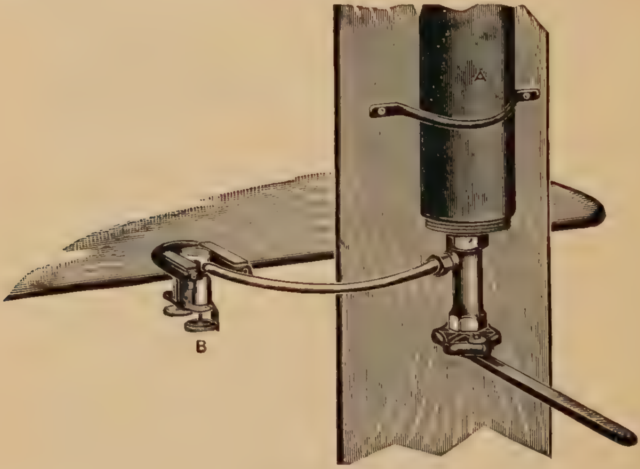


FIG. 46.

If the expense of the ether or carbonic acid gas apparatus is an objection, a very serviceable substitute may be found in the apparatus shown in section in Fig. 45. *A* is a short cylinder closed at top and bottom. *B* and *C* are short tubes opening into the cylinder. A rubber tube is used to connect *C* with a small faucet inserted near the bottom of a pail containing a mixture of ice and salt prepared as for freezing ice cream. A rubber tube attached to *B* carries off the waste water. The cylinder is held by the jaws of the microtome.

The object is first soaked in thin mucilage and then placed on top of the cylinder. In a very short time after the water is turned on, the object is frozen. The process is really very efficient. The cylinder may be purchased from microscope manufacturers or it may be made by any plumber.

In cutting with freezing microtomes the knife should not be moistened in any way. The thawing of the mucilage provides all the moisture necessary. The sections are freed from mucilage by soaking in water, and are mounted in some indifferent medium for temporary examination, or more permanently in glycerine or glycerine-jelly. Or they are passed through the alcohols of gradually increasing strength until the alcoholic strength of the stain to be used (see Appendix for treatment before and after each stain) has been reached, are stained, washed, dehydrated, cleared, and mounted in balsam.

Delicate Animal Tissues. — The directions and cautions given in the preceding chapter to avoid shrinkage in plant specimens might be repeated with emphasis for animal tissues, especially in the case of the more delicate structures. The processes described in this chapter up to this point are not sufficiently delicate for the finest work. Here, as in the case of delicate plant tissues, much care must be exercised in killing, fixing, and hardening the cell-contents, in avoiding the shrinkage due to the transferal from one grade of alcohol to another of widely differing strength, in passing abruptly from alcohol to other media, or to sudden changes of temperature in the imbedding processes. The methods already detailed in the preceding chapter for killing and fixing the cell-contents are equally applicable for use with animal tissues; the methods detailed in this chapter for fixing and hardening animal tissues may, in general, be used with success for vegetable tissues. The solutions of corrosive sublimate, picric acid, osmic acid, Merkel's solution, and the other preparations containing chromic acid, may be especially mentioned as suitable for both animal and vegetable tissues.

Killing. — Small animals, such as worms, may usually be killed in an extended condition by placing them in a small quantity of water and adding alcohol a few drops at a time, repeating at intervals. The process must not be hastened; shrinkage and contraction will surely result if sufficient time is not allowed after each addition of alcohol, or if too much alcohol is added at a time. Small animals may also be killed instantaneously by plunging them into a strong aqueous or alcoholic solution of corrosive sublimate. The solution may be used cold, but a hot solution is better for some specimens. After the solution has acted from ten to twenty minutes, it should be thoroughly washed out with water or with 70 per cent alcohol to which a little tincture of iodine has been added, and the hardening should then be completed in alcohol. The corrosive sublimate solution may be modified by the addition of from 1 to 5 per cent of acetic acid, which will in many cases improve the results. Specimens killed with corrosive sublimate stain well, especially with the carmine stains. Use neither iron nor steel implements in handling the objects. Camel's-hair brushes or wooden toothpicks may be used.

Chromic acid and *Merkel's fluid* are much used in killing. They fix and harden at the same time, but are open to the objection that specimens treated with them are stained with difficulty. As soon as the animals are dead, remove them, wash out the acid very thoroughly with water, and complete the hardening by passing through the higher alcohols, beginning with 70 per cent. Hæmatoxylin is to be preferred for staining in mass; aniline stains for sections. The difficulty of staining objects that have been treated with chromic acid solutions increases with the time which elapses after the objects are removed from the chromic acid solution. Such objects should therefore be stained as soon as possible.

Other killing agents are hot water (150° to 195° F.), chloroform used as a vapor or added in successive small quantities to the surface of the water in which the animals are immersed, and nicotine, which may be used in $\frac{1}{10}$ per cent solution or may be blown into the water as tobacco smoke. As soon as the animals are anæsthetized, a large volume of some fixing agent should be poured upon them.

Fixing and Hardening. — Small animals killed by the processes just enumerated, organs and tissues of larger animals, embryos, and similar objects, must have their cell-contents fixed as nearly as possible in their natural condition immediately after death. A multitude of fixing solutions are in use by different workers. Some are of general application; others are adapted only to special cases. A few only of those recommended for general use by competent authorities will be mentioned here.

Corrosive sublimate, used as already described under "Killing," may be employed. After washing out the corrosive sublimate, place in alcohol of the same strength as the stain to be employed, stain, wash out excess of stain, and complete the dehydration.

Flemming's chromo-aceto-osmic fluid is highly recommended for fixing. The fluid consists of 25 volumes of 1 per cent chromic acid, 10 volumes of 1 per cent acetic acid, 10 volumes of 1 per cent osmic acid, and 55 volumes of water. Lee, in his *Microtomist's Vade-mecum*, recommends the following as the classical method practised by the most advanced workers: Fix in Flemming's fluid from half an hour up to one or two days, wash in water, pass through alcohols beginning with 70 per cent, clear in oil of cedar, imbed in paraffin, cut sections, fix to slide, in series if desired, with Mayer's albumen fixative, remove paraffin, stain with safranin or double stain with gentian violet and eosin, and mount in balsam. If preferred, the object may be stained in the mass with hæmatoxylin.

Picro-sulphuric acid possesses many good qualities as a fixing agent. It should not be used for killing, as small animals die in it in a contracted condition. Animals and tissues that have been killed by some other means may be treated with it in the manner already described in the preceding chapter for its use with vegetable specimens. This fluid is not to be used when one wishes to preserve calcareous portions of objects, as it decalcifies.

Zenker's fluid is also highly recommended. The formula is: Corrosive sublimate, 5 grammes; potassium dichromate, 2.5 grammes; sodium sulphate, 1 gramme; water, 100 c.c. When ready to use the fluid, add 5 c.c. of acetic acid. A large volume of the fluid should be used, and the objects should remain in it from twenty-four to forty-eight hours, acording to their character and size. Wash them thoroughly for several hours in water, then for several hours in three or four changes of 50 per cent alcohol, then continue the washing in the higher alcohols, adding a very little tincture of iodine when the 90 per cent alcohol is reached. In this the specimens should remain from one to two weeks. Thorough washing is then continued to perfect dehydration in absolute alcohol. Specimens that have been fixed in Zenker's fluid allow of the use of a considerable variety of staining fluids.

The foregoing fixing and hardening fluids are sufficient to illustrate to the beginner the general processes that it is necessary to follow in order to secure the best results. Other fixing and hardening fluids are given in the Appendix; but the advanced worker must be referred to special works for the processes that have been found successful in particular cases. The literature on this subject is very extensive. Lee's *Microtomist's Vade-mecum*, Whitman's *Methods in Microscopical Anatomy and Embryology*, the *Journal of the Royal Microscopical Society*, and numerous other scientific journals and monographs may be mentioned as the sources upon which the specialist must draw for suggestions in his work. Each worker, aided by all he can find in the writings of others, naturally develops methods of his own as he acquires experience.

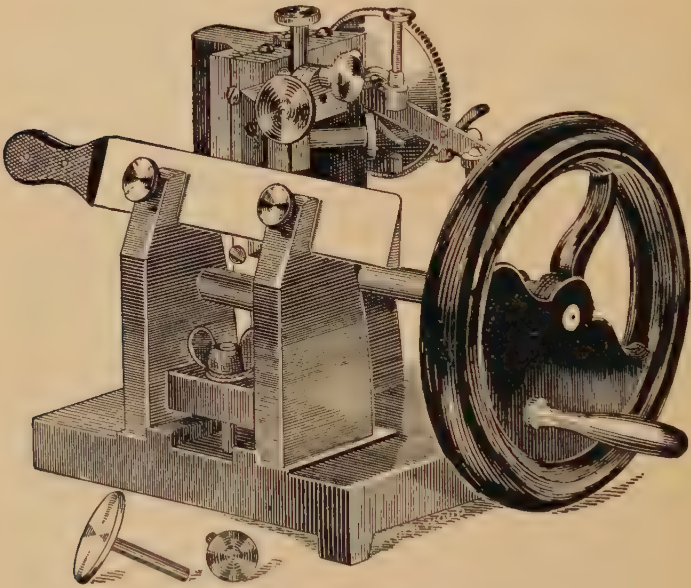
Whatever fixing and hardening fluids have been employed, the objects should not be allowed to remain in them too long, as there are very few fluids that do not produce deleterious effects if their action is prolonged. It is almost invariably best to use the solutions simply to fix the cell-contents and begin the hardening, and to complete the hardening and dehydration in the higher grades of alcohol. If the objects are to be stained in the mass, the staining should be done as soon as the hardening process has been carried as far as the alcohol that corresponds in strength with the alcoholic strength of the stain to be used, and the hardening and dehydration should be finished after the staining. It is a cardinal principle that specimens should not be passed into the higher alcohols and then into alcohols of lower strength to prepare them for stains, if it can be avoided. If the specimens are to be stained on the slide, it is impossible to avoid violating this rule, unless a strong alcoholic stain is used. Strong alcoholic stains are therefore to be preferred for staining on the slide.

The following is the general method of procedure after the objects have been killed, fixed, hardened, and dehydrated by any of the processes: Place the object in a test-tube with a little absolute alcohol; introduce into the bottom of the tube by means of a pipette a little of some clearing agent (oil of cedar, chloroform, xylol, or turpentine); after the object has settled into the bottom of the tube, pour off the mixed fluids and pour on some of the clearing agent undiluted, in which the object should remain several hours or until perfectly cleared; transfer the object to some of the clearing agent that contains all the paraffin it will dissolve; place on the water-bath in this solution and *very* gradually raise the temperature to that of melted paraffin, adding more paraffin as the temperature rises; pour off this solution and pour on pure melted paraffin, taking care that the temperature is no higher than is absolutely necessary to keep the paraffin melted; allow the objects to remain in the melted paraffin from half an hour to twenty-four hours, according to the size and character of the object; imbed in paraffin; cut sections and fix them to the slide, in series if desired, with Mayer's albumen fixative or Schällibaum's collodion fixative; remove paraffin with xylol, benzole, turpentine, benzin, oil of cedar, or chloroform; if the object was stained in the mass, mount in balsam; if the staining is to be done on the slide, pass through alcohols of gradually decreasing strengths until the alcoholic strength of the stain to be used has been reached; stain, wash, and pass through the alcohols of gradually increasing strengths to perfect dehydration, clear with oil of cedar, xylol, benzole, benzin, turpentine, or chloroform, and mount in balsam.

If it is desired to mount in glycerine or glycerine-jelly, transfer the object, after the paraffin has been removed and the solvent of the paraffin has been washed out in alcohol, to a mixture of equal parts of glycerine and alcohol. Allow to stand protected from the dust until the alcohol has evaporated. Transfer to clear glycerine; then mount in glycerine or glycerine-jelly.

If the objects are to be cut in celloidin, they are placed, after they have been killed, fixed, hardened, and dehydrated, in a test-tube with a mixture of absolute alcohol and sulphuric ether. Thin celloidin is introduced by means of a pipette into the bottom of the tube. After the objects have settled into the celloidin, the mixed fluids are poured off and thin celloidin is poured on. They remain in this twenty-four hours; then in thick celloidin twenty-four hours. They are then imbedded. Sections are cut, fixed on the slide, in series if desired, with ether vapor or a small drop of ether, stained unless the object was stained in the mass, washed, dehydrated, cleared in carbolic acid dissolved in turpentine. These objects may also be mounted in glycerine or glycerine-jelly. Place the sections after they are cut in a mixture of equal parts of alcohol and glycerine, allow the alcohol to evaporate, then soak in pure glycerine, and mount in glycerine or glycerine-jelly.

Serial Sections.—It is often desirable to mount sections in series in order that organs may be studied in their entirety. The sections are best cut in chains or ribbons. Special microtomes are adapted to this work, the best of which are perhaps the Cambridge rocking microtome and Minot's automatic microtome. Chains of sections may, however, be cut with any good sliding microtome. No special imbedding masses are necessary, but it is essential that the melting-point of the paraffin used bear a correct relation to the temperature of the room in which the sections are cut. The paraffin should be just soft enough so that the edges of the sections will stick together as they are cut. Experience will determine the right melting-point for the conditions under which the sections are cut. If the knife is kept well smeared with oil, the edges of the



MINOT'S AUTOMATIC MICROTOME.
Made by the Franklin Educational Co., Boston.
FIG. 47.

sections will more easily stick together, and there will not be so much tendency for the sections to stick to the knife. If, however, the paraffin is just right, the oil is unnecessary. Some workers imbed in hard paraffin and coat the paraffin block with a softer paraffin. It is generally necessary to guide the first sections on the knife with a camel's-hair brush or other instrument to start the chain. The knife should be very sharp, and its edge should be set at right angles to the direction of motion and should be parallel to the nearest edge of the paraffin block. The paraffin should be trimmed quite close to the specimen, and the opposite edges of the paraffin block should be parallel. The sections should be cut with quick strokes and as rapidly as the microtome can be worked. Suitable lengths of the chain of sections are removed and fixed on the slide in definite order with Mayer's albumen fixative or Schällibaum's collodion fixative.

If it is desired to mount celloidin or collodion sections in series, they may be fastened to the slide in the usual way with ether vapor, or Weigert's method may be followed. The glass slips on which the sections are to be mounted are coated with collodion by pouring thin collodion on to the centre of the slip and allowing it to flow over it and dry. As fast as the sections are cut, they are arranged in order on a narrow strip of tissue paper which is kept moist with alcohol to prevent the sections from drying up. When the series has been arranged, the strip of paper bearing the sections is inverted over the centre of the collodionized glass slip and pressed gently down. The sections adhere to the film of collodion. Absorb all the alcohol possible with filter paper and allow a little more collodion to flow over the sections. The series of sections will now be held fast in the double film of collodion. The film, which carries the sections, is easily set free from the glass slip by soaking in water. The film can then be put through all the processes necessary for staining, dehydrating, clearing, and mounting without in the least disturbing the series of sections. The different processes consume somewhat more time than with sections not collodionized; this is especially true of clearing. In mounting, a little ether vapor or a minute drop of ether will, of course, fix the film to the slip.

In mounting serial sections, it is a matter of much convenience always to follow a particular method of arrangement, from right to left, left to right, up, or down the slide, and always to begin at the same point. This will avoid much work in writing labels and will avoid the confusion that will inevitably result if some definite system is not followed. Large rectangular cover-glasses are most suitable for serial mounting.

CHAPTER X.

CHEMICAL CRYSTALS.

THE preparation of chemical crystals, either for temporary examination or for permanent mounting, is both easy and attractive. A very large number of chemical substances crystallize in most beautiful forms from solutions. The same chemical often gives crystals of very different forms when treated in different ways. For many substances it is necessary only to make a solution in water, place a drop of the solution on a cover-glass or glass slip, and allow it to evaporate at the ordinary temperature of the room. The solution may be made in a small test-tube or in a watch glass. Very small quantities only of the chemicals and of the solvent should be used, as but a small drop of the solution is needed. Another method of making the solution is to place a drop of water or other solvent on a cover-glass or glass slip, add a few particles of the chemical, and apply gentle heat.

Different forms of crystals are obtained from solutions of different strengths. If saturated solutions do not give good crystals, especially if the results are massive and coarse, try weaker solutions. Some of the most delicately beautiful crystals form from very weak solutions. Other solvents than water may be used, as alcohol, ether, chloroform; and the greatest variety of interesting forms of crystals results. The forms also vary according as the evaporation is rapid or slow. The best results are obtained from some substances by retarding the evaporation, by placing the cover-glass with the drop of solution on it in a damp, cool cellar, until the crystals are formed; sugar is an example of such substances. Crystallization may also be retarded by adding to the solution gelatine in varying quantities. A few strips of gelatine are placed in a test-tube with a considerable volume of water and, after soaking some minutes, are dissolved by the application of heat. A little of this solution is poured into another test-tube or into a watch glass, and the chemical under treatment is dissolved in it. The crystallization is allowed to take place at the ordinary temperature of the room. Many of the common chemical salts give excellent results when treated by this method.

Some solutions need to be evaporated by the application of artificial heat or by exposure to the direct rays of the sun. Potassium chlorate, potassium chromate, potassium dichromate, potassium oxalate, potassium permanganate, potassium iodide, sodium chloride, cobalt chloride, ammonium nitrate, copper nitrate, oxalic acid, cadmium sulphate, iron sulphate, barium chloride, nickel nitrate, and many other common chemical salts may be dissolved in water and treated by this method.

In other cases no solution is made, but a small amount of the solid substance is placed on a cover-glass and held a few inches above a flame until it melts. The cover-glass is then withdrawn from over the flame and, upon cooling, the substance recrystallizes in its own water of crystallization. Salicin, santonin, and benzoic acid are substances which give especially fine results by this method.

For temporary examination a solution of the chemical salt is made by one of the methods described, and a few drops placed on a glass slip previously somewhat warmed. The slip is placed on the microscope stage, slightly inclined, and the crystallization watched with a low power, — two inch, one inch, or three-fourths inch objective, — under polarized light. If crystallization does not begin within a few minutes, the slip is again slightly warmed. A cover-glass should not be placed over the crystals in this examination.

Interesting and instructive crystals are also formed by filling shallow cells, made by cementing rings to glass slips, as described in Chapter VI., with a saturated solution of the chemical under examination and setting aside, protected from the dust, until the crystals are formed. A thread laid diametrically across the cell, just touching the liquid, will often cause the crystallization to start along this diameter and so be in a convenient part of the cell for observation. The crystals formed in a considerable quantity of liquid are much more characteristic of the substance than the tabular crystals, which result when a thin layer of liquid crystallizes on a flat surface. For this reason no study of the crystallization of a substance is complete until an examination has been made of the crystals formed in the "mother liquor." The crystals may be permanently preserved in these cells. After the crystals have been formed, fill the cell even full of the saturated solution of the substance used, place the slip on the turn-table, and run a fresh ring of shellac cement on the outer edge of the cell, breathe on a cover-glass, place it over the cell, press it gently down, and see that there are no air bubbles. When dry, finish as already described for cell mounts. Slides thus prepared should be handled carefully, to prevent displacement of the crystals by sudden jars.

For permanent mounting it is better, as a rule, to form the crystals on the cover-glass rather than on the slip. A cover-glass is cleaned, slightly warmed, a drop of the solution of the chemical in water, gelatine-water, alcohol, ether, chloroform, or other solvent, is placed on the centre, and the crystals are allowed to form. It is not to be expected that every trial will give results worthy of preservation, but a little patience will meet with sure reward. The crystals may be mounted in various ways. Carpenter recommends castor oil as the best preservative. Balsam mounting is much easier, and is most excellent for those substances that will stand the balsam without dissolving. The following substances, crystallized by this method, are permanent in pure balsam: potassium chlorate, potassium ferrocyanide, potassium ferricyanide, potassium oxalate, potassium chromate, potassium dichromate, copper sulphate, iron sulphate, tartaric acid, ammonium oxalate, borax, salicin, cane sugar, and doubtless many others.

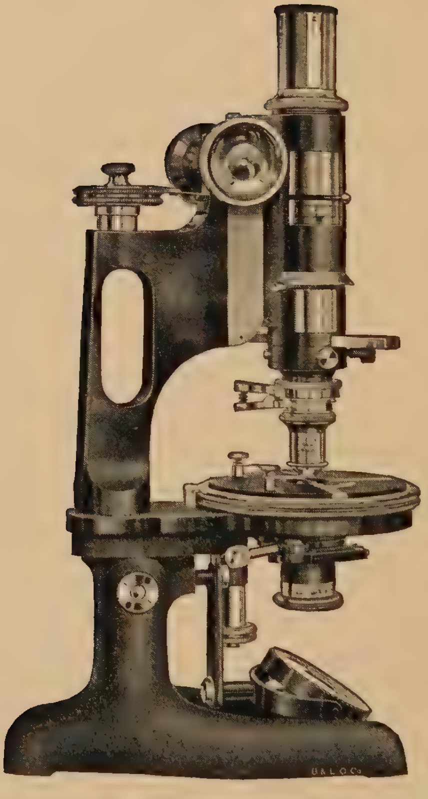
In mounting, a drop of pure Canada balsam is placed on the centre of a glass slip, the cover-glass on which the crystals have formed is inverted over it, centred, and gently pressed down, care being taken to disturb the crystals as little as possible. The balsam must be allowed to harden at the ordinary temperature.

A method applicable with a few substances, and giving most beautiful results, is to place a small drop of balsam on a slide, heat until the volatile matters are driven off, then add a little of the solid chemical, and heat again until solution of the chemical in the balsam is effected, put on a warm cover-glass properly centred, and set it aside to cool. This method possesses the advantage of effecting the mounting with a minimum of trouble, and the added advantage that the slide may, immediately upon cooling, be freed from the superfluous balsam and receive the finishing rings of cement. Sulphonal and salicin give excellent results by this method.

Most chemical crystals may be preserved in dry mounts. A shallow cell is necessary, best formed of shellac cement, though paraffin cells are popular for this purpose. It is best to have on hand a quantity of cells ready prepared, of various depths, and to use in each case one that is but little deeper than the thickness of the crystals to be mounted. Crystals of deliquescent and efflorescent substances, if mounted dry, must be mounted and thoroughly sealed immediately after they are formed and before the air has had time to act upon them.

In the examination of crystals polarized light should be employed. Even crystals belonging to the cubical system are brought out more clearly in some positions of the Nicols than they are in ordinary light. These crystals do not polarize, of course, unless, as sometimes happens, the film is subjected to a strain while the crystals are forming. Crystals of other systems polarize with endless variety of colors. The selenite plate accompanying the polarizing apparatus, or in lack of this a selected film of mica, placed on the stage under the slide, gives a colored field which aids in bringing out the forms and colors for crystals which do not happen to be of the right thickness to polarize well of themselves; but the use of the selenite detracts rather than adds to the interest of other crystals which are of suitable form and thickness to give brilliantly beautiful colors without adventitious aid. In the course of the examination, the rotating Nicol should, of course, be turned to obtain different color effects, and to enable the observer to watch the change of each color to its complementary.

It is hardly necessary to give an extended list of chemical substances from which crystals may be formed. Many nitrates, some sulphates, most chlorates, oxalates, citrates, and tartrates form polarizing crystals.



Bausch and Lomb Petrographical Microscope.
Fig. 48.

CHAPTER XI.

ROCK SECTIONS BY SIMPLE MEANS.

Within recent years the study of the microscopic structure of rocks has come to be recognized as an important feature of geological investigation. The microscope helps in the determination of the soundness and of the purity or impurity of minerals, and aids in deciding upon the nature and character of the impurities. Before the application of the microscope to rock sections, chemical analysis was the only means of determining the composition of rocks. In many instances where chemical analysis fails, the microscope decides with certainty on the mineral composition; and in the case of certain minerals, nearly all that we know of their origin has been learned from the microscopical study of their sections. It is true that the microscope cannot settle all questions, but it has already largely superseded chemical analysis where the end in view is simply the determination of the mineral ingredients; and in most cases where a quantitative determination of the minerals is to be made by chemical processes, the microscope lends important aid in making the preliminary qualitative determination. The value of the microscopical study of rocks is being more and more recognized.

The practical use of the microscope for these purposes involves a vast amount of study — study of great interest, and which, on account of the newness of this line of research, offers peculiar attractions. It is not the presumption of this chapter to give an exhaustive treatment of petrology, but to state simple methods for the preparation of thin sections of rocks for microscopical study, with a few hints at the methods of studying these sections.

Specialists have devised special apparatus for the preparation and examination of rock sections, which is quite out of the reach of most private workers on account of the expense involved. A great deal may be done with the usual microscopical outfit. Many sections of rocks yield very interesting results when examined by ordinary light; for the examination of most rocks polarized light is, however, essential. A revolving stage gives an additional means for their study. A plate of selenite or mica gives a colored field which, in many cases, is a help in bringing out the characteristic features of minerals. This outfit of apparatus is all that is really necessary for a large amount of work in this interesting line of microscopical investigation.

If one has the use of a lathe, slices of rocks may be sawed by means of a thin disk of iron or copper charged at its edge with diamond dust, and these slices may then be ground down on emery wheels. This method of working, however, involves too much expense for most private workers.

Very simple means suffice for much interesting and instructive work in grinding rock sections. All that is needed is a smooth piece of iron — an old stove cover or a piece of smooth sheet iron will do — some coarse emery, say, No. 120, or even coarser for many rocks, some flour of emery, bits of window glass, and some Canada balsam.

For a specimen to begin with, granite is good. Select a granite that is firm and sound. With a hammer chip off a piece about half an inch square and as thin as possible. Place a little of the coarse emery on the iron plate, together with a little water. Holding the specimen firmly with the fingers, grind one side flat. Wash off the coarse emery, and continue the grinding with flour of emery in water on a plate of window glass until the surface is quite smooth. Wash the specimen again and dry it. Now place a few drops of Canada balsam on a piece of window glass about an inch square, and heat to drive off the volatile portions of the balsam. A spring clothes-pin makes an excellent holder for this operation. The balsam, after cooling, should be quite hard and firm, but not brittle. Care should be taken not to let the balsam take fire. Place the specimen, smooth side down, in the warm balsam, and press down upon it gently until the balsam has cooled, seeing that no air bubbles are enclosed between the specimen and the piece of glass. When the balsam is cold, the specimen will be firmly cemented to the glass. Using the glass as a holder, continue to grind until the specimen is reduced in thickness to such a degree that light is readily transmitted through it.

The specimen should be examined at intervals under the microscope to watch the progress of the work. Wash it free of the emery, place a drop of water on it, and cover with a cover-glass. As the specimen becomes thin, care should be taken that the balsam is not worn away up to its edge. If this happens, the specimen may be rapidly broken away. The remedy is to clean and dry the specimen, place a little fresh balsam on it, drive off the volatile matter, and let the balsam set around the specimen again. The latter portion of the grinding should be done in the flour of emery on a plate of glass, as before.

When the specimen has been reduced to the desired degree of thinness, place on it a large drop of Canada balsam, and heat until the object is released from the piece of glass. Then with a needle carefully slide the specimen into a watch glass of alcohol, and let it remain until the balsam and contained dirt have been removed from it, draining off the alcohol and adding a fresh portion once or twice, if necessary. The specimen may now be lifted by means of a camel's-hair brush, and placed on the centre of a microscope slide, dried carefully in gentle heat, and mounted in Canada balsam.

Very brittle specimens are liable to be broken in the process of removal from the glass holder to the alcohol. This may in a measure be guarded against by placing a cover-glass over the specimen after the fresh balsam has been added. Heat and remove specimen and cover-glass together to the alcohol. The cover-glass will also serve to support the specimen in removing it from the alcohol to the slide. See that the section is on the centre of the cover-glass, remove, drain, and dry. Have ready a slide with a drop of balsam on its centre, invert the cover-glass and specimen over the balsam, and centre. In the case of excessively brittle specimens, it is best to omit the washing in alcohol. Remove the specimen, supported by a cover-glass, directly to a glass slip on which a drop of balsam has been previously placed.

The amount of material available for the microscopical study of rocks is unlimited. The dark rocks are, in general, so opaque that they must be ground to extreme thinness unless the sections are to be examined by reflected light; but any of the lighter colored stones yield good results. A cabinet of minerals affords numerous specimens for instructive study. Fossils often yield most beautiful specimens. Silicified wood, for instance, though very hard and requiring much patience in grinding, amply compensates for the labor by revealing the minute plant structure as plainly as a piece of a growing tree. Pieces of silicified wood are sometimes found which, by a blow from a hammer, cleave into thin films, ready to mount for the microscope. The study of the microscopic structure of fossil plants, though until recently but little attention was paid to it, is now recognized as a valuable adjunct to the study of growing plants. Indeed, it is only by the study of fossil specimens that the historical development of modern plants can be investigated.

All rocks of crystalline structure afford much interesting material. When a section of such a rock is viewed by means of polarized light, the variegated coloring is very beautiful. As the analyzer is turned, each color changes to its complementary. The labor bestowed in grinding rock sections is amply repaid if no further object is sought than to obtain beautiful and interesting objects for a cabinet.

In the study of the sections of a crystal in a rock section, it is to be remembered that the form of the section will depend upon the direction in which the section was cut. A cubical crystal might, for instance, give any of the following shapes: an equilateral, an isosceles, or a scalene triangle, a square, an irregular quadrilateral, or a parallelogram. The same rock section will often give several different sections of crystals of the same mineral. At the most, the study of a small number of sections of the same rock will usually give quite a definite idea of the shapes of the crystals that it contains. In some cases, however, the crystals are much changed in shape by compression in the rock mass, so that the study of the crystalline structure is made complicated.

If the revolving stage of the microscope has a graduated edge, and the tube of the instrument has centring screws, so that its axis may be made to pass accurately through the centre of the stage, the angles of the crystals may be measured. An eye-piece with crossed hairs is used. The point of intersection of the hairs is made to coincide with the vertex of the angle to be measured, and one of the hairs is made to coincide with one side of the angle. The stage is then revolved until the same hair coincides with the other side of the angle, and the number of degrees through which it has been turned is read from the graduated edge of the stage. The measurement of the angles affords valuable means for the identification of crystals.

The presence or absence of cleavage is another means of identifying minerals. The cleavage lines come out very plainly under the microscope, even when they cannot be made out in the massive structure with the unaided eye. The angle formed between the cleavage planes determines at once several different minerals. It is to be remembered that the direction in which the section is cut relatively to the face of the crystal modifies the size of the angle, so that it is important to determine in what direction the section is made with reference to the face of the crystal before relying upon the angle to decide what the mineral is.

In studying sections of crystals under the microscope, the lack of purity is very noticeable. Minerals which to the unaided eye seem to be perfectly pure are often found to contain large amounts of other substances, either crystalline or amorphous. Crystals enclosed within crystals are very common; these crystals are sometimes large enough to be determined; sometimes so small that identification is impossible. Cavities are also frequently found which contain a small bubble of water, or of liquid carbonic acid, or a small cube of common salt, which has crystallized from the supersaturated salt water that was enclosed in the rock at the time of its formation.

As we have seen in Chapter III., polarized light furnishes the means for determining whether a substance is singly refractive or doubly refractive. If some portions of a rock section, viewed in polarized light, do not restore the light when the Nicols are crossed, and do not give color effects when the analyzer is turned, or when the stage is rotated, it is at once known that they are singly refractive or isotropic. If, under these conditions, the light is restored or color effects are produced, these portions of the section are doubly refractive or anisotropic.

The singly refractive portions of the section are either amorphous, or are crystals of the isometric system, or are sections of crystals of other systems cut perpendicularly to an optic axis. Amorphous substances are readily distinguished from crystalline substances, from the fact that the latter almost invariably show either a definite polygonal outline or cleavage lines. Isometric crystals may be distinguished from crystals of other systems cut perpendicularly to an optic axis by means of convergent polarized light. To obtain the convergent polarized light, petrographical microscopes are provided with a convex lens, to be mounted just above the polarizer, or with some other means of converging the light. If a section of an isometric crystal, cut in any direction, is viewed in this light, nothing more is noticed than when ordinary light or parallel polarized light is used. Sections of crystals belonging to the tetragonal and hexagonal systems cut perpendicularly to the vertical axis, which, as we have seen, act like isometric crystals in parallel polarized light, when viewed in convergent polarized light, show rings of prismatic colors and a distinct black cross when the Nicols are crossed. This cross remains stationary when the crystal is revolved. It is changed to a white cross as the analyzer is revolved, while at the same time the colors of the rings change to their complementaries. These basal sections of hexagonal and tetragonal crystals are further distinguished by the outline of the section, which is a polygon of some multiple of three sides for the hexagonal crystals, and of four or eight sides for tetragonal crystals. There is but one direction in hexagonal and tetragonal crystals, viz. that parallel with the vertical axis, along which parallel polarized light can pass without modification, and it is only when convergent polarized light passes in this direction that the rings of prismatic colors and the black cross result. Parallel polarized light passing in any other direction through crystals of these systems is again polarized. The field is dark when the vertical axis of the crystal corresponds to the planes of vibration of either Nicol, light in other positions of the crystal, the Nicols being crossed. Color effects are produced as the crystal is rotated or the analyzer turned. These crystals are uniaxial.

In the case of crystals of the orthorhombic, monoclinic, and triclinic systems, there are two directions in which parallel polarized light passes unmodified. In all other directions it is doubly refracted. These crystals are biaxial. A section perpendicular to either of the optic axes behaves like an isotropic substance in parallel polarized light. In convergent polarized light, more or less elliptical rings of prismatic colors are seen; but instead of a black cross, as in basal sections of crystals of the tetragonal and hexagonal systems, when the Nicols are crossed, a single bar of black will be seen. A further distinguishing characteristic is the fact that when the crystal is revolved the black bar revolves in the opposite direction.

If a section of a biaxial crystal, cut perpendicularly to the line which bisects the acute angle formed by the optic axes, is viewed in convergent polarized light with the Nicols crossed, a series of elliptical rings of prismatic colors with two black hyperbolas will be observed in one position of the crystal. If the crystal is rotated, the hyperbolas give place to a black cross with unsymmetrical arms. If the analyzer is turned, the black hyperbolas change to white, and the prismatic colors to their complementaries.

Any section of an orthorhombic crystal, placed so that any one of the three crystallographic axes is parallel with the planes of vibration of the light, is dark between crossed Nicols. Some sections of monoclinic crystals are dark between crossed Nicols when a crystallographic axis is parallel with the planes of vibration of the light, while others restore the light. In the latter case, if the section is revolved through a certain number of degrees, differing in different crystals, the field becomes dark. Sections of triclinic crystals are never dark between crossed Nicols when a crystallographic axis is parallel with the planes of vibration of the light. On turning the crystal through some degrees, the field becomes dark. The number of degrees through which the crystal must be turned in these two cases is a valuable aid towards determining the crystal. The position of the crystallographic axis can usually be determined by the outlines of the crystal or by cleavage lines.

The determination of the system to which a crystal belongs is, of course, an important step towards its identification. In some cases this determination is quite easy;

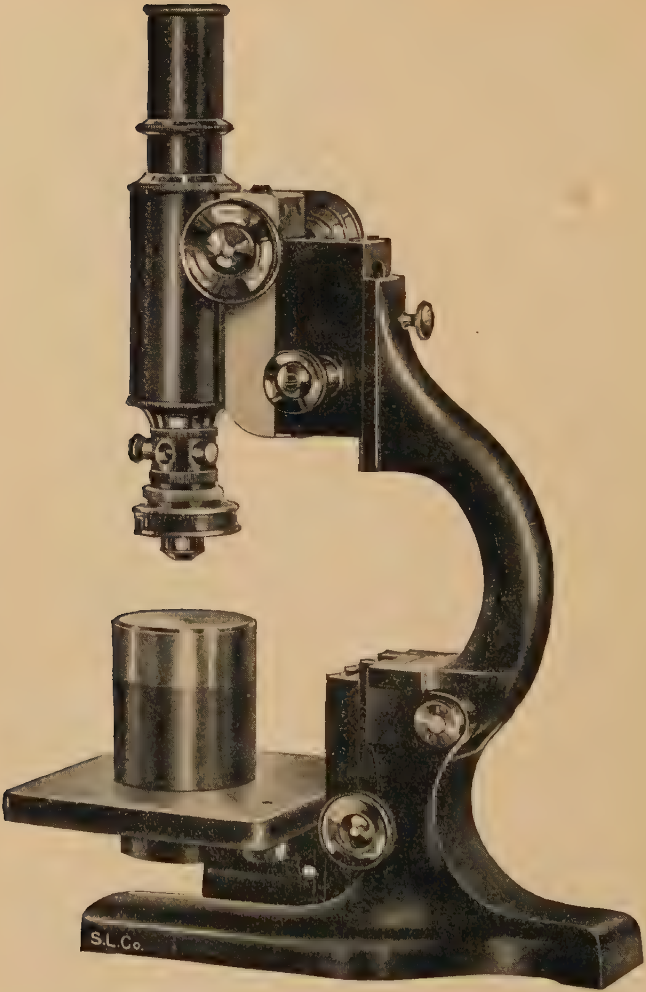
in other cases it is hardly necessary to say that it is most difficult. Systematic study of the crystals in rocks presupposes extensive training in crystallography, calls for the keenest powers of observation, and the highest exercise of the reasoning faculties. What has been here said is intended merely to suggest to the interested beginner some of the methods employed in the microscopical examination of rock sections.

Cleavage lines, angles of crystals, outlines of crystals, determination of the system to which crystals belong, enclosure of crystals and amorphous substances in crystals, cavities containing air, water, or liquid carbonic acid, effect on the light, color of the crystals, association of minerals, are, then, some of the characteristics relied upon in the identification of the components of a rock.

Rolling Sand. — Although not strictly belonging under the heading of this chapter, a slide of rolling sand is properly classed with rock specimens. Such a slide is not very difficult of preparation, and is a most interesting addition to one's collection. Clean quartz sand from the seashore or the beach of a pond or stream is required. A cell of such depth that the sand is free to move after the cover-glass is in position is used. The mounting fiuid is glycerine slightly diluted with water.

Place a small quantity of the sand in a cell, fill the cell full of the fluid, and see that there are no air bubbles clinging to the sand or to the edges of the cell. Run a ring of shellac cement around the outside of the top of the cell, and put the cover-glass in position. See that no air bubbles are enclosed. Clean and finish the slide in the manner already described for glycerine mounts.

For examination, slightly incline the stage of the microscope, having the Nicols crossed. Hold the slide edgewise for a moment until the sand runs down to one side. Place the slide in position on the stage with the sand uppermost. As the sand now rolls slowly down (it appears, of course, to roll up hill), a most magnificent display of color changes is seen as the different positions of the grains of sand bring different thicknesses of the quartz between the Nicols. The specimen is very showy.



Spencer's Metallurgical Microscope.

Fig. 49.

CHAPTER XII.

THE STUDY OF BACTERIA.

In the study of bacteria the microscope plays only a subordinate part, yet without it this study would be impossible. The numerous methods of preparation, culture, and isolation of bacteria lead up to an examination under the microscope. Some account of such methods in their simpler forms would seem then to have a place in these pages. Much may be done with powers of the microscope not higher than four or five hundred diameters. The processes employed may be learned, and very definite ideas of what bacteria are, and the means used by specialists in their investigations, may thus be acquired. For the more satisfactory study of such preparations, however, a substage condenser and the very highest powers of the microscope are necessary, used with the highest degree of manipulative skill. It is not to be expected that the finer points of detail will be seen except by these means.

The beginner is cautioned to remember that bacteria are the minutest organisms in nature, and that they can be seen under moderate powers of magnification only by the closest observation. In looking for them, focus sharply with the fine adjustment and expect to see exceedingly minute rods, spheres, and curves, or, in the case of filamentous forms, very fine segmented threads. These are all highly transparent, unless they have been submitted to the action of some staining fluid. They may easily be overlooked, but a little persevering observation will cultivate the eye to detect them.

The name *bacteria* is applied to countless numbers of forms of vegetable organisms. They exist everywhere. They are the active cause of putrefaction and of fermentation. The decay of all animal and vegetable substances is occasioned directly by the rapid multiplication of different kinds of these organisms. Many chemical changes in organic substances are due to their agency. The souring of milk, for instance, is caused by the action of bacteria, which convert the sugar of the milk into lactic acid, and this into butyric acid and other products. The development of flavors in the ripening of cheese is brought about through the action of bacteria. It is believed that bacteria play an important part in the stomach and intestines in the digestion of food.

Under favorable conditions the multiplication of bacteria is enormously rapid. As long as they can obtain an abundance of food material of the right nature, they multiply by fission; but if food material fails, or the conditions for this kind of development are otherwise not right, a clear round or oval spot appears in each individual organism of many of the species. This is the resting-spore. It continues to live without nourishment after the rest of the organism has disappeared; it resists drought and cold. These spores, in the dry condition, are taken up by the air, and are afterwards deposited on all exposed surfaces. When they are brought in contact with suitable nourishing material they spring into life with great vigor.

The reproduction is effected by the splitting of the cells. Hence the name, Schizomycetes, or fission-fungi. There are, however, good reasons for believing that they do not belong with the fungi. Various forms occur. All are enclosed in a structureless gelatinous membrane. One general division includes minute spherical cells which divide by a wall running across in one direction, or by walls crossing one another; these receive the general name of Micrococcus. Another division, including straight, rod-like forms, receives the name Bacillus. If the rods are very short and thick, with somewhat rounded ends, the name Bacterium is employed. Slender, long rods are called Leptothrix; thick, long rods, attached at one end of the filament to some supporting object, are called Beggiatoa. Crenothrix is a name used for a form of large rods having about them an especially distinct gelatinous envelope. Branching forms are called Cladothrix. The cells of the rod-like forms multiply by first increasing in length, and then throwing a partition transversely through the middle of the cell, the new cells formed repeating the process indefinitely, giving rise to chains of cells connected by a highly transparent membrane. The individual cells are so small that the minute structure is very hard to study. So far as is known, each cell is filled with protoplasm of uniform composition, but differing somewhat in different species. The belief that protoplasm is present is based upon the fact that only the more opaque portions are stained when iodine is run under the cover-glass. Of the coiled forms, those that are short, stout, and have but few coils are called Spirillum; those that are long, slender, and have many coils are called Spirochæta. Vibrio is a name applied to loosely curved and segmented forms. In each of these general divisions are innumerable individual forms, many of which have been studied and given particular names. It is to be remarked, however, that there is more or less of confusion in the use of these names in the literature of bacteriology.

The prevailing colors of bacteria are gray, yellowish white, and white. Some species are bright, and are readily recognized in masses as red, blue, green, or brown. In some cases the coloring matter exists in the protoplasm; more frequently it is found in the envelope of cellulose-like material which surrounds the protoplasm. The coloration arises from the power which bacteria possess of chemically separating the substances in which they thrive, and of forming new compounds, or upon their power of absorbing ingredients from the surrounding substances. Crenothrix and Cladothrix are of a distinct brown color, due to the oxide of iron thus absorbed from the water in which they thrive. The yellow color of Beggiatoa is due to sulphur, which has in a similar way been separated from the sulphur water in which these bacteria grow. The blood-like drops which form on bread and some other substances when left in a moist atmosphere under suitable conditions of temperature are simply masses of bacteria, the *Bacillus prodigiosus*, which have developed from germs deposited from the atmosphere. The blue color which sometimes under favorable conditions develops in milk is explained in a similar way. The green color which develops on cheese is another example. It is characteristic of bacteria as an order of plants that they are devoid of chlorophyll. Yet there are exceptions to the rule,—at least some forms give the characteristic blue color when treated with iodine solution, showing that starch, the product of chlorophyll, is present. The color is in some cases an important aid towards the identification of the bacteria.

In connection with color may be mentioned the phosphorescence seen at times on the ocean and on decaying wood. It is caused, in some cases, by different kinds of bacteria, which have the power of separating phosphorus from some of its compounds.

Some forms of bacteria are endowed with the power of motion, or at least have this power at certain stages of their existence; other forms are incapable of moving. It is probable that in the case of some of the moving forms the motion is effected by the contractions of the protoplasm of the cell. In other forms there are hair-like appendages, either from the ends or sides of the cell. These cilia, or flagella, are believed to consist in some cases of protoplasm, and to be organs of motion; in other cases it is believed that the cilia are merely prolongations of the membrane enveloping the cell, and that they have nothing to do with the production of motion. These appendages are naturally difficult to make out, but the number of forms possessing them is now known to be very large.

The observer is cautioned to remember that almost all finely divided solid matters suspended in a fluid exhibit a peculiar oscillatory motion called the Brownian movement. The study of the Brownian movement made in Chapter IV. should be recalled, as many kinds of bacteria exhibit this movement. Careful observation will readily enable one to distinguish between the progressive movement from place to place of the really motile bacteria and this purely mechanical vibratory movement. The hay bacillus, described later, affords good material for the study of both these movements. Another movement about which one might be mistaken is the motion of Vibriones. A filament of Vibriones is shaped like a corkscrew, and the movement is believed to be a revolution on the axis of the filament. Under the microscope it appears by an optical illusion to be a snake-like, undulatory motion. In the case of Spirochæta and Spirillum, the serpentine motions are believed to be real.

Many forms of bacteria are perfectly harmless. In fact, they are essential to life either as a direct agent in digestion, as already noted, or by their action in the decomposition of animal and vegetable substances, and the preparation of the soil for the production of plant life. With the air, the water, and with our food, we receive countless numbers of bacteria into our systems, where they are either inert or perform the functions for which they were designed in the economy of nature for our well-being. The investigations of recent years have equally well established the fact that other forms are pathogenic. It now scarcely admits of question that cholera, typhoid fever, diphtheria, consumption, and many other diseases, are caused and propagated by particular bacteria.

With these introductory remarks, which are intended to call the attention of the beginner to the nature and importance of the subject, we will turn our attention to the methods employed in the study of these exceedingly minute organisms. As already intimated, culture methods must be employed in the satisfactory study of most forms of bacteria. The remainder of this chapter will have to do with forms which can be studied without special cultivation, and with those which can be cultivated by very simple means. For culture methods the reader must be referred to the latest books by specialists. The list is very extensive.

Bacteria of the Fur of the Teeth. — Scrape a little of the fur from the teeth, and place it on a cover-glass in a thin, even layer with a small drop of water. Invert the cover-glass on a slide, and examine with the highest power at command. Several forms of bacteria will undoubtedly be found. Minute spheres of Micrococci are clustered together; a spiral form, *Spirilla dentium*, or, as it is sometimes called, *Spirochæta denticula*, darting back and forth with characteristic movements, may be present; possibly the comma bacillus of the teeth, a form curved like a comma (,) and closely resembling the comma bacillus of Asiatic cholera, will be seen in active motion; larger than any of these, straight, rod-like forms will be found, a variety of Leptothrix. If a little iodine solution be run under the cover-glass, the protoplasm in the largest rods will contract sufficiently to show that the rods are segmented. Possibly a blue color, due to the presence of starch granules, will be seen. With the exception of comma bacillus, the bacteria of the mouth cannot be cultivated by the ordinary means.

Putrefactive Bacteria. — Expose to the air of a living-room at the ordinary temperature pieces of moist bread, boiled egg, boiled potato or turnip, for twenty-four to forty-eight hours. Small spots, either translucent or colored, will form. These are colonies of bacteria that have developed from germs deposited from the air upon the substance used.

Transfer a little from one of these spots to a cover-glass with a small drop of water, and examine as already described. Numerous rod-like and spherical bacteria will be seen, many of the rods in active motion.

For further study, boil some green leaves in a flask. Almost any kind of leaves will do. Cabbage or lettuce leaves are good. In another flask boil some ground peas. Filter each solution after an hour, and set aside open at the ordinary temperature. In about forty-eight hours skin-like formations will be seen on the surfaces of the liquids in the flasks. This formation is called a pellicle.

A little of this is examined under the microscope in the manner already described.

Again, soak some hay in water at the ordinary temperature for four or five hours. Filter the infusion into a flask, and boil gently for a few minutes. Plug the mouth of the flask with a wad of cotton-wool, and place it aside at the ordinary temperature for twenty-four or forty-eight hours. A pellicle forms. This is formed by the hay bacillus, *Bacillus subtilis.* The germs of this bacillus are able to withstand boiling for a few minutes. Other bacteria present have probably been killed by this treatment. We have then a pure culture, as it is called, of this variety. The cotton-wool plug is an efficient filter for the air that enters the flask, no germs being able to pass through it. If the boiling was not continued long enough, other forms of bacteria will also be present.

If a bit of the pellicle be examined at the right stage of development, filamentous forms will be seen imbedded in a transparent, gelatine-like substance. In the active, swarming state, the *Bacillus subtilis* divides into filaments of twos, fours, and eights, with flagella at the ends of the filaments. These bacteria are easily stained with gentian.

For still another study, place some Spirogyra, a water plant described in Chapter V., in a flask of water, and allow it to stand until decomposition begins. From time to time examine a drop of the water. Very lively motile coiled bacteria, *Spirochæta plicatalis*, are likely to be found. These bacteria are common in any stagnant water containing decaying organic matter. In the same water the straight, comparatively thick rods of *Beggiatoa alba* may be found. These are filamentous. The protoplasm is granular in appearance, due to the particles of sulphur.

That these granules are sulphur may be proved by allowing the preparation to dry and then running a little carbon bisulphide under the cover-glass. This will dissolve the sulphur, and the granulation will become less distinct.

In all of these cases, the masses taken from the pellicle show the bacteria in the zoöglœa stage. The pellicle is composed of motionless bacteria surrounded by a gelatine-like, transparent substance which is a product of their growth. If we take our specimen at the right time, we have the bacteria in the active, swarming stage, when they are in active motion. At the proper stage of development, we may see the resting-spores. In order, then, to form correct ideas of bacteria, one should make frequent examination, to see them in the different stages of their development.

Cover-Glass Preparations. — In the study of the above, as well as of all other bacteria, it is of advantage to make a so-called cover-glass preparation. A very thin layer of the medium containing the bacteria is placed evenly on a cover-glass. This is best effected by placing a minute drop on a cover-glass and laying another cover-glass on this. The two cover-glasses are now gently rubbed together, and then separated by sliding them apart. They are then dried at the temperature of the room, or by holding them in the forceps some distance above a gas flame, being careful not to scorch the film. The bacteria are now stained by placing on each cover-glass a solution of fuchsin, Bismarck brown, methyl violet, or other staining solution. This is allowed to act from ten to twenty minutes, or longer. The dye is now washed off with distilled water; and, if there has been considerable diffuse staining of the ground tissue, the cover-glass is washed in alcohol. The cover-glass is now dried as before, and a small drop of oil of cedar, turpentine, or xylol is placed upon it. It is then inverted on a glass slip, and is ready for examination.

In some cases double staining is of advantage. After the surplus of the first stain has been washed away, as above, the cover-glass is not dried, but is treated with a contrast stain from ten to twenty minutes, washed, treated with alcohol if necessary, dried, and mounted in oil of cedar, turpentine, or xylol for examination. Fuchsin and Bismarck brown, and Bismarck brown and methyl violet or methyl blue give good results. These stains may be used in water solution or in alcoholic solution, with the exception of Bismarck brown, the alcoholic solution of which deteriorates on standing. A nearly saturated solution in alcohol of the other dyes may be kept on hand and diluted with ten times its bulk of water, and filtered, at the time of using. As a rule, the bacteria take only one of the colors. The object of the second color is to stain the ground tissue, and so set forth the bacteria by contrast.

A cover-glass preparation made as above may be permanently preserved by simply draining off the oil of cedar, turpentine, or xylol, and mounting in xylol or benzole balsam. Do not use chloroform balsam, as the chloroform dissolves the aniline dye. The bacteria are very much more distinctly seen when mounted in balsam than when mounted temporarily in oil of cedar, etc.

A somewhat quicker process and one much used is to fix the bacteria to the cover-glass or slide by passing several times through the flame, using the carbolated white of egg as a fixative when necessary. Then drop on enough stain to cover the preparation and heat until the solution steams or even boils, renewing the stain as it evaporates. Wash with water followed by alcohol or by alcohol to which 1% to 3% of nitric, sulphuric, or hydrochloric acid has been added, if the staining is diffuse. Dry thoroughly over the flame and mount in balsam, or examine without mounting with the oil-immersion objective. A well-made preparation may be used repeatedly, if the immersion oil is washed off with xylol after each use, wiping off the excess of xylol with the ball of the finger.

Hanging-Drop Cultures. — This is a very convenient form of culture for some investigations. Cells are made by cementing rings of glass, rubber, or metal to microscope slides, as described in Chapter VI. When the cultures are to be made, sterilize the cells by washing in the 1: 1000 solution of corrosive sublimate or in absolute alcohol. Smear the top surface of the cell thickly with vaseline. Sterilize a cover-glass in the same way or by passing it through the naked flame. If the bacteria are growing in a liquid, merely place a drop of the liquid on the cover-glass and put the cover-glass, drop downward, on the ring. If the bacteria are on a solid medium, thoroughly mix some of the medium in a small amount of physiologic salt solution (7.5 grams of common salt dissolved in 1000 c.c. of water), place a drop of the mixture on the cover-glass, and invert the cover-glass over the cell, pressing it down gently. The vaseline makes an air-tight joint.

Many of these slides may be prepared at the expense of very little labor. They may be set aside at the required temperature, and studied under the microscope whenever one wishes. The drop of nutrient medium, being attached to the cover-glass, is in a suitable position for examination. If it is desired to keep the preparation moist, a very deep cell may be used, and a drop or two of water that has been sterilized by boiling may be placed in the bottom of the cell.

Another good cell for moist cultures is made by cutting a hole a little smaller than the cover-glass in a piece of thick pasteboard. Soak the pasteboard in boiling water to sterilize it, place it while still wet and hot on the centre of a microscope slide, and immediately place over it a prepared cover-glass with its inoculated hanging drop. To keep up the moisture, wet the pasteboard from time to time in boiled water. If these cultures are to be prolonged for some time, keep the slides under a bell-glass with a dish of water.

The hanging drop is much used in studying the movements of motile forms, spores, capsules, size and arrangement of bacteria.

It is somewhat difficult to focus on hanging drop cultures with a high-power objective, as the bacteria are so minute, transparent and in a transparent medium; also on account of the danger of crushing the cover-glass. It is a good plan to focus on the edge of the drop with a 16 mm. objective; then change to a 3 or 4 mm. objective. If the objectives are parfocal, as they should be, and as they are on the best modern microscopes, slight movements of the fine adjustment screw should now bring the bacteria into focus. The iris diaphragm of the sub-stage condenser should be well closed down, as the bacteria are much more easily seen in subdued light. The concave mirror also usually works better than the plane. It requires great skill to use an oil-immersion objective on hanging drops. The 3 or 4 mm. objective gives sufficient magnification for most observations of this type.

Useful Stains. — No attempt is made in this chapter to detail the numerous processes that have been devised by specialists for the staining and double-staining of bacteria for special purposes. In no department of medicine has so much progress been made. Only specialists writing for specialists can adequately discuss the subject. The number and variations in stains and staining technique to be found in the recent literature of bacteriology is exceedingly large and would fill volumes. A few only of the most common and generally useful stains and processes will be given here.

In all delicate work it is essential to see that the slide and cover-glass are absolutely clean. The best means to accomplish this is to use the soap, Bon Ami, as it is used for cleaning window glass. Cover the slide or cover-glass with a thick paste of Bon Ami, rubbing with the fingers until nearly dry. Let dry completely and rub off and polish with a dry, soft cloth. Stitt says, " This is far better than soap and water, alcohol, ether and flaming combined." To make perfectly sure hold the slide or cover-glass in the flame to burn off any possible film of grease that may remain. If the glass is perfectly clean a small drop of water will show no tendency to cling together, but will spread out into a very thin film over the glass.

In making the smears the process already described may be used, or a *very small* amount of the material to be examined may be rubbed in a *very thin* film over the cover-glass or slide with a piece of glass tubing drawn out to a small diameter or with a wooden toothpick. In making smears from growths on agar or other culture media, place a small drop of distilled water on the cover-glass or slide and rub into this a *minute* amount of the material fished from a colony on the culture. Let the smear dry in air and pass three times through the flame to fix the bacteria to the glass. Another highly recommended method of fixing the bacteria is to place a drop of alcohol on the dried smear, drain off by tipping the cover-glass or slide on its edge against a piece of filter paper. Then hold in the flame to ignite the alcohol and let it burn off.

Solutions of gentian-violet, methylene blue and fuchsin in 95% alcohol may be kept in stock, and small portions may be diluted with ten or twelve volumes of water at the time of use. These three stains will suffice for a large amount of elementary study of bacteria. It will not be out of place here to mention a few modifications of them.

Gram's Method. — This method is much used to distinguish certain bacteria from others of similar appearance. Those bacteria that stain by the method are known as Gram-positive; those that do not take the stain are known as Gram-negative. Two solutions are used. For the first there are several modifications, three of which are here given.

1\. *a.*

Aniline water (Prepared by thoroughly shaking 5 c.c. of aniline oil in 100 c.c. of water. Let stand some hours and filter several times)	100 c.c.
Saturated solution of gentian-violet in alcohol	5 c.c.

This solution does not keep well and must be frequently renewed.

1\. *b.*

Gentian-violet (saturated alcoholic solution)	1 c.c.
Carbolic acid (5% aqueous solution)	10 c.c.

1\. *c.* Weigert's Stain:

Solution 1.		*Solution 2.*	
Gentian-violet	2 gms.	Gentian-violet	2 gms.
Aniline oil	9 c.c.	Distilled water	100 c.c.
Alcohol (95%)	33 c.c.		

These stock solutions keep well. For use add 1 c.c. of solution 1 to 9 c.c. of solution 2.

Stain in any one of the above three stains from two to four minutes, but do not boil. Then apply

2\. Lugol's Iodine Solution:

Iodine	1 gm.
Potassium iodide	2 gms.
Water	300 c.c.

Allow the preparation to remain in this iodine solution until it becomes very dark. Then wash in changes of alcohol until only a faint bluish color remains.

A counterstain of fuchsin or of Bismarck brown dissolved in boiling water may be used. Only the Gram-negative organisms take the counterstain. Gram-positive organisms take the purplish stain of the gentian-violet.

Löffler's Methylene Blue. — This is a good differential stain for a very large number of bacteria. Preparations stain more intensely if slightly heated. The formula is:

Saturated solution of methylene blue in alcohol	30 c.c.
1: 10,000 solution of potassium hydrate in water	100 c.c.

The Ziehl-Neelsen Carbol-Fuchsin Method. — This stain is of primary importance as the best one for the tubercle bacilli, but it is also an excellent stain for many other acid-fast organisms. The most commonly used method for the tubercle bacilli is as follows: Spread the sputum on a glass plate; with the help of a hand lens pick out the small, white, opaque, caseous particles a millimetre or less in diameter and make cover-glass or slide smears of them. Dry in the air and fix by passing three times through the flame or by the burning alcohol method.

Stain in

Carbol-Fuchsin

Fuchsin	1 gm.
Absolute alcohol	10 c.c.
Dissolve and add	
Carbolic acid (5% aqueous solution)	100 c.c.

This stain may be heated in a watch glass until it steams and the prepared cover-glass floated on it, prepared side down, from three to five minutes; or the solution may be used cold, in which case it should be allowed to act for twenty-four hours. The smear is then washed in 95% alcohol containing 3% of hydrochloric acid. The acid removes the stain from all bacilli likely to be present except from tubercle bacilli. Wash in water and dry between filter papers. As a contrast stain, use a saturated solution of methylene blue in water, allowing it to act ten seconds or longer. Wash and dry the preparation between filter papers and above the flame, clear in aniline oil or xylol and mount in xylol balsam.

A method commonly employed by physicians does not give as perfect preparations, as there are more likely to be deposits from the staining fluids. The smear is made on a slide, fixed by passing several times through the flame or by the burning alcohol method, covered with carbol-fuchsin stain, heated to steaming by holding the slide over the flame for one to five minutes, the stain being renewed as it evaporates. There is no danger of overstaining, as the tubercle bacilli resist taking the stain to such an extent that it is probable that all the bacilli in a specimen do not usually take it. After rinsing in water, acid alcohol is dropped on the preparation until the red color is largely discharged. Any tubercle bacilli present will not be affected by this treatment. Rinse in water and cover with an aqueous solution of methylene blue or with Löffler's methylene blue. These stains act quickly, but there is no danger of overstaining. In successful preparations the tubercle bacilli are stained a deep red; other microörganisms and other elements on the slide take the blue stain. Wash in water, dry between filter papers and pass several times through the hot air above a flame to remove the last traces of water, clear in xylol and mount in xylol balsam, or examine without a cover-glass with the oil immersion objective.

Neisser's Stain. — The chief value of this stain is for the examination for diphtheria bacilli, but it is a desirable method for more general application. In examining for the diphtheria bacilli, smears may be made from the sputum or the secretions of the nose, but the best material is obtained from culture growths on blood serum or the white of a hard-boiled egg incubated at about blood temperature.

Two solutions are used:

Solution 1.

Dissolve .1 gm. of methylene blue in 2 c.c. of alcohol. Add this to 5 c.c. glacial acetic acid in 95 c.c. of distilled water and filter.

Let this stain act on the preparation for one minute. Wash in water.

Counterstain in

Solution 2.

Dissolve .2 gm. Bismarck brown in 100 c.c. of boiling water and filter.

Let this stain act about thirty seconds, wash in water, dry between filter papers, clear in xylol and mount in xylol balsam.

The polar granules of the diphtheria bacilli, a dot at either end of the bacillus and sometimes others along the organism, take a deep blue color; the remainder of the bacillus takes a brown color.

Capsule Staining. — The staining of capsules is difficult and requires experience. The following methods are all highly recommended by good authorities.

Hiss's Method for Temporary Mounts. — Stain for thirty seconds in gentian-violet (saturated solution of gentian-violet in alcohol diluted with an equal volume of water). Wash in 0.25% solution of potassium carbonate in water and examine in the same. Use no clear water at any stage.

Hiss's Method for Permanent Mounts. — Stain in gentian-violet or fuchsin (5 c.c. of the saturated solution of the dye in 95% alcohol plus 95 c.c. of water), steaming over the flame for a few seconds. Wash in 20% solution of copper sulphate in water. Remove excess of copper sulphate with filter paper. Use no clear water. Dry thoroughly and mount in balsam.

Muir's Method. — Stain the dried and fixed smear for 30 seconds in carbol-fuchsin, heating to steaming. Wash slightly in alcohol and then in water. Treat the preparation with a mordant composed of 2 parts of a saturated aqueous solution of mercuric chloride, 2 parts of a 20% aqueous solution of tannic acid, 5 parts of a saturated aqueous solution of potash alum. Wash thoroughly in water, cover with 95% alcohol, letting it remain about a minute, wash again thoroughly in water. Counterstain in methylene blue for about 30 seconds. Wash in water and apply two changes of alcohol, clear in xylol and mount in xylol balsam.

Rosenow's Method. — Cover the nearly dried smear with a 10% solution of tannic acid for 20 seconds. Wash in water and dry with filter paper. Stain with the Gram gentian-violet, steaming for about a minute. Wash in water. Treat with Lugol's iodine solution for a minute. Decolorize in 95% alcohol. Counterstain in an alcoholic solution of eosin. Wash in water, dry and mount in balsam.

The sputum of a person suffering from pneumonia affords good material for capsule staining. If the capsules are well developed, the Gram stain gives excellent results. Other micrococci likely to be present in the sputum are Gram-negative except streptococci which may easily be confused with pneumococci, as the colonies are almost identical in appearance and the streptococci also form capsules at certain stages of development. Harmless streptococci are very commonly present in the mouth. It is believed by many that the pneumococcus is a modified, malignant form of streptococcus, and it is sometimes designated *Streptococcus pneumoniae.*

To study capsules without making stained preparations, make hanging-drop cultures and examine with the iris diaphragm of the sub-stage condenser well closed.

Spore Staining. — Float the prepared cover-glass face down on carbol-fuchsin for five minutes. Wash *slightly* in alcohol to which 2% of hydrochloric acid has been added, followed by water. Counterstain for 30 seconds in a freshly prepared solution of methylene blue, wash in water, dry and mount in balsam.

Möller's Method. — Cover with chloroform for two minutes, wash in water, cover with 5% solution of chromic acid for two minutes, wash in water, stain with carbol-fuchsin heating to boiling, without washing decolorize in 1% sulphuric acid for half a minute, wash in water, counterstain for 15 to 30 seconds with methylene blue, wash in water, dry and mount in balsam.

If successful, the spores will be stained red, and the bodies of the cells will be blue. The best material from which to secure forms showing spores is obtained from twenty-four hour agar cultures, but ordinary smear mounts sometimes show them.

Flagella Staining. — The Muir-Pitfield method, recommended by Stitt, is as follows: The material should be from an 18–24 hours growth on agar. With a platinum loop add the growth to the top of a test tube full of sterilized water until a faint turbidity is seen. Place in an incubator at 37° C. for two hours. Apply a loopful from the top of the test tube to a cover-glass. Place in the incubator for several hours. Then treat with a mordant composed of 10 parts of a 10% aqueous solution of tannic acid, 5 parts of a saturated aqueous solution of mercuric chloride, 5 parts of a saturated aqueous solution of alum, and 5 parts of carbol-fuchsin stain. The precipitate which forms in preparing this mordant is allowed to settle and the clear solution above is used. The mordant does not keep well. After the mordant on the cover-glass has been gently steamed for one minute, wash thoroughly in water, dry and stain for one minute, steaming, in a freshly prepared stain composed of 10 parts of a saturated aqueous solution of alum and 2 parts of a saturated alcoholic solution of fuchsin or gentian-violet. Wash thoroughly, dry and mount in balsam.

Good books for the more advanced study of bacteria are: Jordan's *General Bacteriology;* Stitt's *Practical Bacteriology, Blood Work and Animal Parasitology;* Prescott and Winslow's *Elements of Water Bacteriology;* Race's *The Examination of Milk.* Many other excellent books could be added.

CHAPTER XIII.

PHOTOMICROGRAPHY.

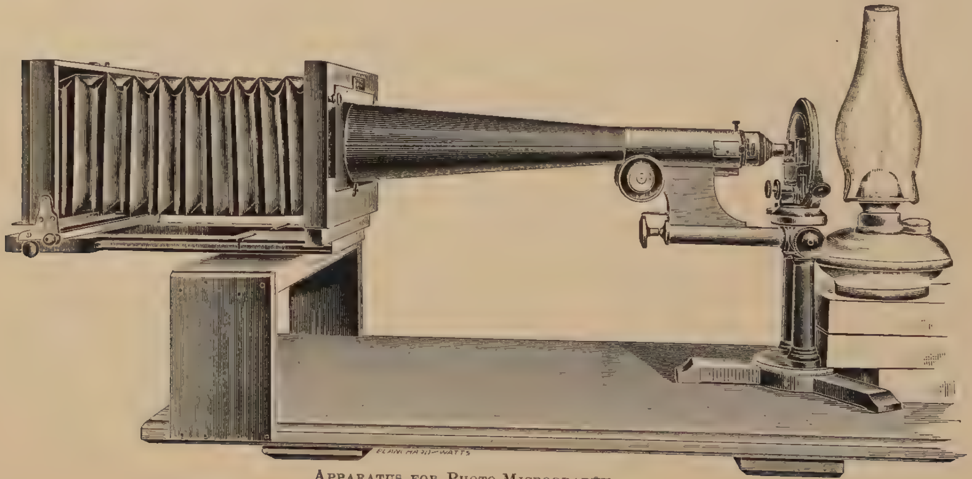
THE necessity for true representations of objects as seen under the microscope, and the tediousness and difficulty of making accurate free-hand or camera lucida drawings, have naturally called the attention of microscopists to the photographer's camera as a means of securing, with a small expenditure of time and labor, multiplied pictures of microscopic objects. The rapid advances made in the art of photography in very recent years have rendered the accomplishment of this, to a certain extent, within the reach of every microscopist. For certain classes of objects, and for the purpose of fixing the details of structure in the mind of the student, the free-hand or camera lucida drawing must be made, but for many classes of objects, and when it is desired to have numerous reproductions, the photomicrograph is largely superseding the laborious processes of the draughtsman.

The art of photomicrography offers to the private worker a most delightful avocation; for the teacher and lecturer, it provides the means to secure material for illustration in botany, zoölogy and related subjects; the physician can use it to make permanent records of important facts in regard to histological and bacteriological conditions that may present themselves in his practice; the expert may furnish the manufacturer or merchant information that is of great value in his business.

The manufacturers of microscopes have placed on the market apparatus for taking photomicrographs, some with all the refinements to be desired by specialists in various fields; others less complete and less serviceable. Very few private workers would feel warranted in purchasing such outfits. Much may be done with apparatus that can be largely constructed by anyone having only ordinary skill in the use of tools, or the cost would be slight to have it made by a carpenter. This apparatus is thoroughly efficient for the practical work of the laboratory. It is the purpose of these pages to describe simple and inexpensive apparatus and processes to be used with the usual and ordinary equipment of the microscope and to illustrate by reproductions of photomicrographs taken with such apparatus the character of the work that can be done with this equipment.

Apparatus. — Any microscope with a tube large in diameter and an ordinary plate photographic camera, omitting the lens, are all that is necessary for low-power work. A camera for a 4″ x 5″ plate with kits for the use of a 3¼″ x 4¼″ plate may be used, as these are the sizes best adapted for the work, and involve much less expense than larger sizes. In fact a 3¼″ x 4¼″ camera is large enough for practically every purpose, would do away with the inconvenience arising from the use of kits, and for this reason possesses advantages over a larger size. If larger pictures are desired, they may be made by the simple process of enlarging, and the results will usually be better than if made directly by greater amplification, as most ordinary objectives break down in performance when the amplification is too great.

Arrangement of Apparatus. — For the simple arrangement shown in Fig. 50, the microscope may be used with or without the eye-piece. By trial determine which gives the better image on the ground glass of the camera for the particular specimen to be photographed. The mirror and sub-stage fitting are removed or swung to one side. The tube is inclined at right angles to the supporting pillar. The



APPARATUS FOR PHOTO-MICROGRAPHY.

FIG. 50.

camera is used without its lens, the microscope objective or objective and eye-piece being alone used. A board four feet long by ten inches wide, placed on any firm table, is used as a support for both camera and microscope, — an arrangement which gives great steadiness. At one end of the board, a rigid, upright support is placed, to which the camera is firmly attached, at such a height that the tube in which the photographic lens is usually inserted is exactly opposite the end of the inclined tube of the microscope when it is placed in position on the board. The tube of the camera should be larger in internal diameter than the tube of the microscope. The junction of the two tubes must be made light tight. This may be accomplished by winding a black cloth around the tubes. See also page 199. The light to be used is an ordinary kerosene lamp, properly placed, at a distance of about ten inches from the stage of the microscope, so as to bring the flame onto the optical axis with the edge of the flame towards the microscope. The proper way to do this is to remove the camera and remove the eye-piece from the microscope. Then adjust the lamp until the image of the flame is seen, on looking along the microscope tube, at the exact centre of the back lens of the objective. Now restore the camera to its position and see that the circle of light centres exactly on the ground glass.

Low-power objectives, as a rule, give better photographs when used without an eye-piece. High-power eye-pieces should never be used with achromatic objectives, but low-power eye-pieces may be used to advantage with high-power objectives. If no eye-piece is used, care must be taken to avoid *flare* spots, i.e., spots of uneven lighting caused by reflections from the inside of the microscope tube. If flare spots occur, line the inside of the tube with black velvet.

The simplest and at the same time the most satisfactory method of making the exposure is merely to insert a black card between the source of light and the microscope as near the microscope as practicable, or, if a very low-power objective is being used, between the objective and the object on the stage, to cut off the light while the dark slide is being withdrawn from over the plate, removing the black card for the period of the exposure and then replacing it to cut off the light while the dark slide is put back to cover the plate. The black card should be mounted on a lever so that it can be thrown in and out of position and leave both hands free to handle the dark slide. The room should be dimly lighted and *no extraneous light* should be allowed to fall on the microscope during the exposure.

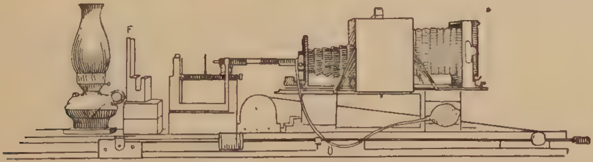


FIG. 51.

F is a support for a ray filter. See Fig. 66.

Fig. 51 shows another simple arrangement. Here a small Leitz III microscope which has no joint in the pillar is supported in the horizontal position at the height of the optical axis of the camera. An ordinary plate camera having an automatic shutter is shown in the illustration. The photographic lenses are removed, the shutter being left in position on the camera. A brass tube about two inches long with a thread is screwed into the shutter in place of the front photographic lens, thus providing a means of effecting the exposure. Almost any automatic shutter with a sufficiently large opening may be adapted to this use when an eye-piece is used.

With the apparatus shown in Figs. 50 and 51 an Abbe condenser may also be used without any further change in the arrangement. The light should be about ten inches from the Abbe. The ordinary Abbe condenser cannot be used with very low powers.

When the apparatus has been arranged as described, light the kerosene lamp and put out all other lights in the room. See that the circle of light on the ground glass is perfect. Place a mounted microscopic object on the stage of the microscope, and fasten it in position with the spring clips. Adjust the objective to about its working distance, and move the ground glass of the camera until the image of the object is seen on it of such size as desired. The image is much more distinctly seen if the ground glass is well shaded from the light. By changing the position of the ground glass and regulating the focussing of the objective to correspond, the object may be projected onto the ground glass variously magnified from a few diameters to the full capacity of the ground glass.

When an evenly lighted field has been obtained, focus sharply, first with the coarse adjustment, then with the micrometer screw. In the final adjustment of the focus, the image on the ground glass should be viewed with a hand magnifying glass or with a focussing glass.

All is now ready for the dry plate, but before exposing read the sections of this chapter on exposure, orthochromatic plates and ray filters.

For work with the low powers up to and including the 16 mm. objective, the arrangements shown in Figs. 50 and 51 are perhaps the most satisfactory that can be used by a beginner. There is no steadier and more reliable light than that of an ordinary kerosene lamp, and it is of sufficient intensity for the powers mentioned arranged in the simple ways described. Good results may also be obtained by using incandescent electric lights, but the adjustments are very difficult to make and to a considerable extent are secured only by lucky chance rather than by the application of any principles. It is advised that no time be wasted in experimenting with them. The trouble with such lamps is that overlapping images are formed by the filaments, and these give uneven illumination.

Some workers commend the incandescent gas burner highly. The inverted type is preferred. It is good with very low powers, where the light source must be large to fill the objective. With this burner the light is less steady than that of a kerosene lamp, as it varies considerably with the fluctuations of the gas pressure. Deterioration of the mantle is also a source of trouble. Any changes in the intensity of the light of course cause changes in the time of exposure.

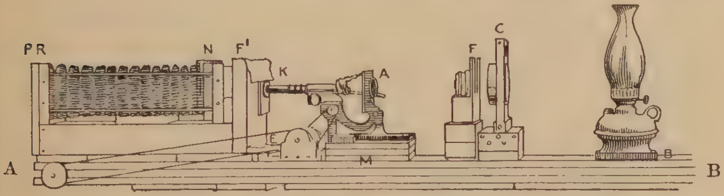


Fig. 52.

C is the auxiliary condenser, a plano-convex lens, four inches in diameter and about five inches focal length, placed about fourteen inches from the Abbe condenser, *A*, with the plane side turned towards the light. The lamp is about ten inches from the auxiliary condenser. *F* is a support for ray filters.

While the simple arrangements already described are all that are necessary for low-power work with a kerosene lamp as the source of light, if higher-power work with more intense lights is to be done, an arrangement that will allow of the easy interchange of different pieces of apparatus should be adopted.

A desirable arrangement is shown in Fig. 52. *AB* is an optical bed six feet long and eight inches wide. To prevent warping and secure greater rigidity this is made double with cleats between the parts.

Along the middle of the entire top of the optical bed a straight strip, four or five inches wide and rising about half an inch above the surface, is fastened. This strip we will call the guide strip. Every piece of apparatus placed above it — base for the camera, base for the microscope, bases for lenses, lamp and ray filter — fits closely over this guide strip, and each piece may be removed and replaced independently of the others, thereby providing for a number of different arrangements of apparatus and adaptations to different uses to be described hereafter. The height of the optical axis is determined with reference to the height of the lamp to be used.

The base on which the camera is mounted slides up against a stop at *E*. Through this stop are driven two wire nails parallel with the top surface of *AB* which engage in holes bored in the base holding the camera when the camera is moved into position from left to right on the guide strip, which hold the front securely in position and which allow the camera to be removed and replaced at will. In taking the picture the left end of the camera may be held fast to the optical bed, if necessary, by means of an ordinary clamp. The heavier the construction the better, as the apparatus will be steadier and less liable to suffer from vibrations. It requires but an instant to slide the camera to the left and remove it from the optical bed. When replaced, it takes exactly the same position as before.

The camera shown in Fig. 52 is wholly of home construction even to the bellows, but with the exception of the camera back, for which the back of a 4″ x 5″ camera was utilized. The front end of the camera is fastened rigidly to the base. The camera back is supported on each side by two brass curtain rods secured in uprights, *P* and *N*, both of which are firmly attached to the base of the camera. These rods pass through the piece *R*, so that the camera back may be moved to and fro freely. When the desired position is reached, the camera back is held in position by small bolts arranged as follows. The piece *R*, Figs. 52 and 53, is made in two parts. The nuts of the bolts *BB* are imbedded between these pieces and remain fixed in position, the bolts working through the nuts and pressing against the rods which pass through holes in the left-hand piece. The construction is of course duplicated on the other side of the camera. At *F′*, Fig. 52, is a slot to receive a ray filter. This is covered with a cloth flap. Near the front is the handle of a simple flap shutter arranged on the inside of the camera. This shutter is covered with black cloth and effectually shuts out the light. Such a shutter is, however, open to the objection that in its operation vibrations may be set up in the apparatus at the critical instant when the exposure is being made. An automatic shutter is much to be preferred. At *K*, Fig. 52, a metal flange and a short tube larger than the tube of the microscope are secured.

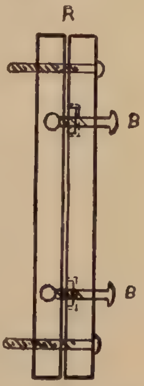


FIG. 53.—ARRANGEMENT FOR HOLDING THE CAMERA BACK.

A fitting to place on the upper end of the microscope tube to make a connection that will exclude the light may be purchased. Such a light-excluder is very easily made. Take a section one and one half or more inches long of a pasteboard tube, the internal diameter of which is about one quarter of an inch greater than the external diameter of the tube on the front of the camera. Fit a thin flat cork (bung) to one end, cutting a circular hole in it to fit tightly over the upper end of the microscope tube. Place this tube with open end towards the camera on the tube of the microscope. When the camera is moved into position, the tube on the front passes inside the tube of the light-excluder, making a connection that effectually shuts out the light. The tube on the camera should not, in this operation, in any way touch or come against the light-excluder or the microscope, as disturbances of the arrangements would be caused.

A light-excluder of this construction is especially desirable when no eye-piece is used, as the tube on the camera may be made sufficiently large not to cut off any of the light coming from the objective. The illumination on the ground glass will then be limited only by the diameter and length of the microscope tube.

In this camera the distance from the object on the stage to the ground glass is thirty-six inches when the camera is fully extended, and the length of the bellows allows for a considerable range in magnification.

Another camera used interchangeably on the same optical bed consists of a simple box ten inches long and six inches square. The camera back above referred to fits one end. To the front is attached a tube four inches in diameter and about three inches long. When the camera is brought into position this tube passes inside a light-excluder four and one quarter inches in diameter placed on the eye-piece end of the microscope tube. To give greater camera extension several tubes, four and one quarter inches in diameter at one end and contracted to four inches at the other end and of different lengths, are provided. These tubes are made of thick paper and are, of course, well blackened on the inside. Several of these tubes may be telescoped together and used as one. When greater camera length is wanted, the camera is drawn back and a tube of the proper length to give the magnification desired is slipped at one end over the tube on the camera front and at the other end into the light-excluder on the microscope and light-tight connection is again established. By this simple and inexpensive arrangement very efficient provision may be made for camera extension up to the limits at which ordinary objectives and eye-pieces work well.

Owing to the large size of the connecting tube and the long distance that may be had between the stage and the ground glass, this arrangement is especially useful when no eye-piece is employed.

The camera, adjusted to the height of the lamp, is placed in position over the guide strip on the optical bed so as to run easily back and forth. A support, *M*, for the microscope, grooved to fit over the guide strip, is made of the right height to bring the tube of the microscope when turned to the horizontal position at the same height as the tube of the camera. An arrangement of pulleys is placed on the front of the optical bed to control a side fine adjustment. Fig. 51, in which the optical bed is reversed in position, shows the usual long rod arrangement used to control a fine adjustment at the top of the pillar of the microscope.

After centring the apparatus to be described later, pieces of wood to serve as stops are placed on the microscope support at the front and the back of the foot of the microscope to insure its return to the same position. The microscope may then be removed at will. When an object is found that it is desired to photograph, the microscope is returned to its position, where it will register as before.

As to the illuminant, if it can be afforded one of the electric lamps described later with a lens corrected for spherical aberration should by all means be obtained; a ray filter will take care of the chromatic aberration. With such a lamp the adjustment is very simple, a much wider range of work can be done, and the difficulties of the worker are reduced to the minimum.

The second choice would be to order from a maker of microscopes a corrected lens of proper focal length to use with a kerosene lamp, stating what use is to be made of it. A corrected lens may be of much smaller diameter than an uncorrected one. The expense is not great.

The third choice would be a bull's-eye lens. Various writers claim good results with even the highest powers with uncorrected plano-convex lenses (bull's-eyes). With such lenses of the highest quality excellent photographs can be made. Many of these lenses are, however, utterly worthless for photomicrography, and an attempt to use them will lead only to disappointment and discouragement. Here again it is best to order the lens from a reliable maker of microscopes, stating what it is to be used for and the illuminant that is to be employed.

Either the corrected or uncorrected lens may be obtained mounted on a stand with means for adjusting vertically and with centring screws. They are less expensive if obtained fixed in short metal tubes, in which case they are used as indicated in the following description. This description is intended for those who do not feel that they can afford the expense of corrected lenses and a more powerful light.

The reason for using an auxiliary condenser of any type is that stronger illumination is secured, permitting of a wider range of work and shorter exposures. Equally good photomicrographs can be made with the first two simple arrangements already described, but the range of work is limited to lower magnifications.

At *C*, Fig. 52, is a support for the auxiliary condenser. In this case a plano-convex lens, part of the condenser of an old-fashioned Marcy's Sciopticon, is used. It is four inches in diameter and of five inches focal length. With this particular lens even illumination — the first requisite for photomicrography — is obtained with some combinations, but not with all. It is not of the highest quality.

Owing to the shape of the uncorrected lens, the rays passing through the peripheral portions are refracted at different angles from those passing through the central portion and come to a focus at different points. A blurring of the image results. For similar reasons color fringes are produced about the image. A diaphragm to cut off the peripheral portion of the lens is therefore necessary to correct in a measure both the spherical and chromatic aberration. An iris diaphragm that may be adjusted to the best position is much to be desired, but diaphragms made by cutting circular openings in pieces of tin may be used. Several of these with openings from one to two inches in diameter should be made, and the one that gives the best results should be selected. These are easily made by marking with dividers a circle of the diameter of the metal fitting of the auxiliary condenser on a piece of thin tin and with a narrow, sharp chisel cutting out the circle, leaving three or four projecting pieces to be turned at right angles to the surface of the tin and serve to hold the diaphragm on the metal fitting of the lens. In the same way draw a circle of the diameter of the desired opening in the diaphragm and cut around it. In the subsequent pages several other diaphragms to be made in the same manner will be mentioned.

The centre of the lens must be brought *exactly* onto the optical axis of the microscope. It is therefore necessary to have means for adjusting it from side to side and also vertically. Fig. 54 shows a front view, i.e., a view towards the microscope, and Fig. 55 shows a side view, of the construction used. A hole of the proper size to take the lens is cut in a square piece of board, A. This board has tongues, cut with a matching plane, on the right and left sides, which run up and down in grooves, indicated by the dotted lines, cut in the upright pieces *BB*. The head of a bolt about three inches long is imbedded in the piece *C*, which is fastened to the piece *A*. The bolt passes through a hole in the piece *D*. By turning the nut *E*, the lens is raised or lowered. The uprights *BB*, Fig. 54, are secured at the bottom to the piece *F*, as shown in Fig. 55, which is a cross-section of Fig. 54. The piece *F* has tongues on both sides and slides from side to side of the optical axis in grooves cut in the pieces *GG*, Figs. 54 and 55. This movement from side to side is controlled by two small bolts arranged on the principle described in Fig. 53. In Fig. 55 the end piece carrying the bolt has been removed to show the interior construction. Fig. 54 shows the end pieces in cross-section at *H H*. By loosening one bolt and tightening the other, the piece *F* is

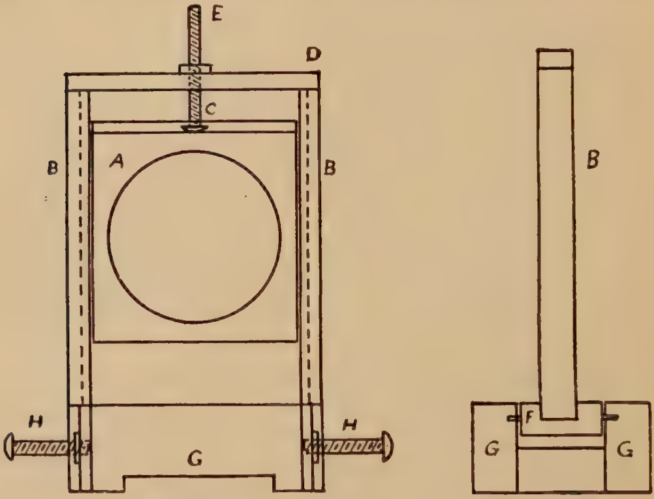


Fig. 54. Fig. 55.

Support for Auxiliary Condenser.

moved from side to side and, when the right position is found, held securely.

All the wood and tin parts of the apparatus are painted a dead black with a paint made by mixing lamp black with dilute shellac.

Adjustments of the Apparatus. — To set up the apparatus, first place the microscope in position, removing the eye-piece and swinging the mirror and Abbe condenser to one side. In the place of the eye-piece, put a diaphragm of tin with a pinhole opening at the centre. Place a kerosene lamp with the edge of the flame turned towards the microscope about two feet from the Abbe condenser. Sight through the pinhole eye-piece and adjust the lamp until the image of the flame is at the exact centre of the back lens of the objective. Then swing the Abbe condenser into position, adjusting it to its approximate focus. If the Abbe is correctly centred, the image of the flame will still be seen in the exact centre of the back lens of the objective. If it appears not to be properly centred, see page 281.

Now, remove the objective, close the iris diaphragm of the Abbe condenser to a pinhole opening and place the auxiliary condenser in position, covering it with a pinhole diaphragm. By means of the vertical and lateral adjustments of the lens support adjust the lens until the light passes brightly through the pinhole opening in the diaphragm over the lens, the pinhole opening of the Abbe condenser and the pinhole eye-piece. Unless the centre of the lens is exactly on the optical axis of the microscope, no light will pass. If a second lens is to be used as auxiliary condenser, as in Fig. 58, remove the first lens with its support and perform the same operation with the second lens. Then restore the first lens to its position and see that the light passes at its greatest brilliancy.

These adjustments are a little troublesome on the first trial and demand a little patience on the part of the worker, but when once made the different parts need no further attention, and any one or all may be removed from the guide strip and replaced at will.

Restore the eye-piece and a low-power objective to the microscope, open up the diaphragm of the Abbe condenser, remove the pinhole diaphragm from the auxiliary condenser and replace it with a diaphragm having an opening about two inches in diameter.

Focussing the Auxiliary Condenser. — Either parallel or convergent light may be used. Brighter illumination will be obtained with convergent light; it is easier to use parallel light. If parallel light is to be used, place the auxiliary condenser with its plane side towards the lamp at a distance of ten or twelve inches from the Abbe condenser. Place the flame at the principal focus of the auxiliary condenser, and the light will pass in nearly parallel rays from the auxiliary condenser to the Abbe. Exactly parallel light is impossible of attainment owing to the defects of the lens, but light sufficiently near to parallel for the purpose is easily obtainable. Move a piece of white paper back and forth between the auxiliary condenser and the Abbe and adjust the lamp until the beam of light is practically of the same size for all positions of the paper. The edge of the flame should be towards the microscope both for parallel and for convergent light.

For convergent light the flame should be placed about two feet from the Abbe condenser, and the auxiliary condenser must be focussed. Move it back and forth between the lamp and the Abbe condenser until a sharp magnified image of the flame is projected onto a piece of paper inserted in the slot in the Abbe condenser provided for the blue glass. Two points will be found, if the auxiliary condenser is moved slowly from near the Abbe condenser to near the light, when a clear image of the flame will be projected onto the paper. In one the lens will be nearer to the Abbe than it is to the lamp. In this case the image of the flame will be *smaller* than the flame itself. This is *not* the correct position. Move the lens towards the flame until the image is again sharp and larger than the flame. When the lens is in this position and the image the sharpest that it is possible to obtain, the position is correct.

Remove the slip of paper and close down the iris diaphragm of the Abbe condenser until the image of the flame completely covers its opening. Remove the eye-piece and sight along the tube of the microscope. If the adjustments are correct, the back lens of the objective should be about two thirds filled with light.

The next step is to determine whether or not even illumination is secured. The proper way to do this is to focus some object sharply onto a paper screen supported in the vertical position in the approximate position of the ground glass of the camera. The lamp should be covered with a hood. This may be improvised by inverting a tall cracker tin over the lamp, cutting a small hole for the light to pass to the microscope and providing for good ventilation at the back. Now remove the object from the stage and study the illumination. Bring the Abbe condenser close up to the slide and slowly move it away until the illumination is at its brightest. The correct focussing of the sub-stage condenser is of the greatest importance. If any unevenness of illumination can be detected, it is useless to try to take a photograph. A considerable amount of study of the adjustments to secure even illumination, to be repeated in making any new combination of apparatus, is profitable.

Now place the camera in position and, using a low-power objective and no eye-piece, observe the circle of light projected onto the ground glass. If all the adjustments have been correctly made, the illumination should be perfectly even and the circle of light should centre exactly on the centre of the ground glass. When a photograph is taken, if any unevenness of illumination can be detected, go over the adjustments again. The importance of making all these adjustments with a great degree of accuracy cannot be too much emphasized, for without this accuracy uneven illumination will result, which will give badly lighted and uneven photographs.

Apparatus for Use of the Electric Arc Lamp on the Direct Current. — Until very recently, the difficulties in the way of high-power work have been almost insuperable for the average worker on account of the lack of a suitable light of sufficient intensity and steadiness. The use of the sunlight, always unreliable and requiring a specially darkened room and expensive apparatus, was out of the question. So also was the lime light, on account of the cumbersome and expensive apparatus. The arc electric light was available for those who were fortunate enough to have the direct current and was, and is now, the best light for high-power work when dense ray filters are used. But on the alternating current it is simply impossible on account of the fluctuating

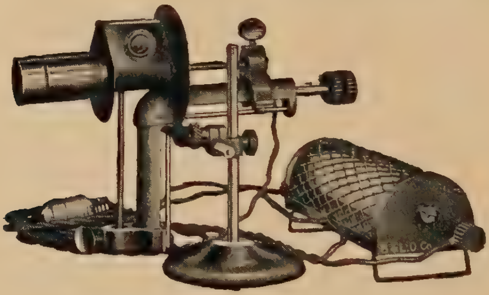


FIG. 56. — BAUSCH AND LOMB ARC LAMP.

nature of the current. The spot of greatest brilliancy on the positive carbon shifts suddenly and with great irregularity so that no dependence can be placed on it for photomicrographic work. It is advised that no time be wasted in experimenting with it unless the direct current can be used.

If the direct current is available and an arc lamp is to be purchased, it is better to buy of a manufacturer of microscopes a lamp requiring four or five amperes, especially designed for the purpose, enclosed in a light-tight casing and fitted with a condensing lens corrected at least for spherical aberration and adapted to the lamp. Such a corrected lens is a *sine qua non* for the most perfect work. No other auxiliary condenser is required.

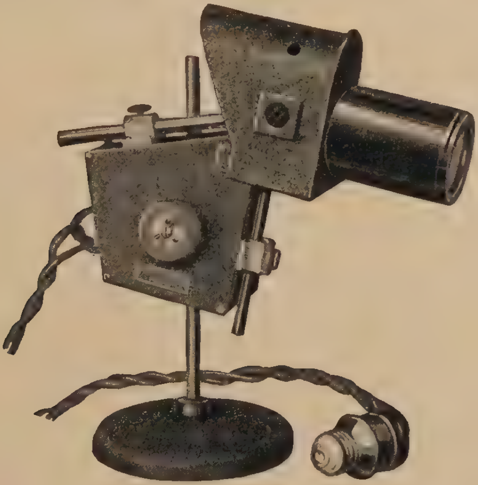


Fig. 57. — Spencer Electric Arc Lamp.

It may be that some have an arc lamp such as is used in a stereopticon and may wish to try it out, using the lenses of the stereopticon as an auxiliary condenser. If these lenses are of the best quality, satisfactory results may be expected; otherwise, not. A study of the apparatus will at any rate pay the beginner.

For high-power work the arrangement is precisely the same as that shown in Fig. 52, except that the image of the light source must, by adjusting the positions of the lamp and lens, be made nearly broad enough to cover the back lens of the sub-stage condenser. A cooling tank is also necessary to prevent possible injury to the microscopic preparations or the lenses of the microscope.

The cooling tank is of simple construction. The sides and bottom are made of wood. The ends toward the microscope and the auxiliary condenser are made of pieces of clear glass, five inches square, placed at a distance of two or three inches from each other. The wood parts should be given several coats of white lead and oil, each coat being allowed to dry thoroughly before the next is applied, and finally a coat of dead black. The glass ends are then cemented in position with white lead putty i.e., putty made by working up dry white lead in linseed oil, adding just enough whiting to give the proper consistency. The tank is filled with water that has been well boiled to drive out the air. If the water is not boiled, air bubbles will collect on the glass ends and obscure the light.

For medium and low-power work another arrangement is shown in Fig. 58. It differs from the preceding only in that two plano-convex lenses are used instead of one. When properly centred and focussed a bright unmagnified image of the light source is formed on a piece of paper placed over the diaphragm of the sub-stage condenser.

The position of the cooling tank shown in Fig. 58 is the best one, as spherical aberrations due to the glass sides are less likely to be introduced if their position is where the light can pass through them in parallel rays. The two lenses may, however, be telescoped together and held by one lens support, the cooling tank being placed on the side towards the microscope. They may also be left on the stereopticon hood, if the microscope and camera can be adapted to this position. If the stereopticon has a partially corrected condenser, the probability of securing evenly lighted photographs is better. If a kerosene lamp is used, the cooling tank is omitted.

If suitable lenses are used, this is a satisfactory arrangement. An Abbe or other sub-stage condenser must be used.

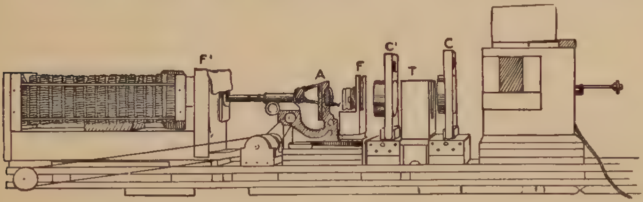


FIG. 58.

C′ is a plano-convex lens placed at its focal distance from the Abbe condenser, *A*, with its plane side towards the Abbe condenser. *C* is another plano-convex lens with its plane side towards the lamp. The lamp is placed at the principal focus of *C*. *T* is the cooling tank. The ray filter is placed at *F* or *F′*. A diaphragm with an opening of one and a half to two inches is placed on the side of *C* towards the tank.

When the adjustments for either of these arrangements have been made, remove the camera from the optical bed and set up the paper screen and practice focussing on it until a perfectly even illumination is obtained. Too much emphasis cannot be laid on the fact that practice of this sort best tends to give thorough mastery of the adjustments and understanding of the apparatus. This is also a very convenient way to run over microscopic objects to select specimens or fields of specimens to be photographed.

It is to be reiterated that these two arrangements for the use of the arc light on the direct current with uncorrected lenses for auxiliary condensers are advised only in case the small arc lamp and corrected lenses cannot be afforded. The lamps shown in Figs. 56 and 57 with lenses corrected for spherical aberration are the types most commonly used in this country. No other auxiliary condenser is then necessary, and if the arc lamp requires a current of only four or five amperes, as is usual with such lamps, the cooling tank may be dispensed with.

Apparatus for Use with the Electric Light on the Alternating Current. — Several electric lamps have within the last few years become available for use on the alternating current. The point-o-lite, as it is known in England where it originated, or tungsarc, as it is designated in this country, is a small automatic arc enclosed in a bulb, a small ball of tungsten being the light source. It is very steady and admirably adapted to photomicrography, but requires from six to ten times the exposure necessary with the small arc lamp. Without a rectifier it can be used only on the direct current. The Bausch and Lomb Optical Company supply a small rectifier for the 110 volt, 60 cycles current, making this lamp available for the alternating current. A special starting and controlling resistance is necessary for either type of current. It is somewhat expensive.

Another lamp is the 6-volt, 108-watt, 18-ampere, 100-candlepower Mazda lamp. A transformer must be used to reduce the house current to six volts. It is recommended for medium and low-power work only, as the filament is too coarse to give homogeneous light with the high powers.

Another lamp, and the first to be used for photomicrography, is the 6-volt, straight-coil tungsten filament, 24-watt, 4-ampere, 36-candlepower Mazda. With a transformer to reduce the current to six volts it can be used on any house circuit. It has much less intensity than either of the two just mentioned, and yet it suffices for most low-power photomicrography and for *some* high-power work. The writer purchased one of the first of these that were put on the market, and with it the high-power photomicrographs reproduced in this book were made. The light is absolutely steady and requires no attention during exposure, — a quality that greatly commends it. With low magnifications it meets all requirements; with high magnifications the time of exposure with the dense ray filters is too long to be considered possible, and ray filters in pairs cannot be used at all when the lamp is placed between thirty and forty inches from the Abbe condenser, as it must be to project an image of the lamp filament sufficiently large to cover approximately the full opening of the iris diaphragm of the Abbe condenser. In photographing objects that require great resolution, the conditions just stated *must* be met. With objects requiring less resolution, the lamp may be placed nearer the Abbe, but there is then danger that the numerical aperture of the Abbe, and consequently of the objective, will be so far cut down that diffraction fringes will be formed around the edges of the object. This lamp, while it serves well with the dry objectives, is not to be recommended for work with the oil-immersion objective, as combinations of ray filters that are required for perfect

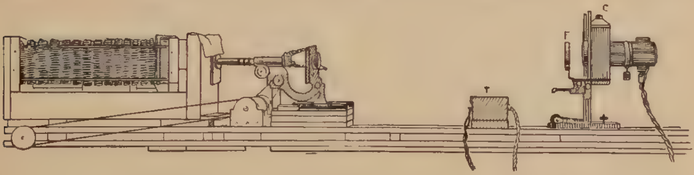


FIG. 59.

Bausch and Lomb illuminating apparatus with 24-watt lamp and camera previously described. The auxiliary condenser is at *C*. *F* is a support for a ray filter. *T* is a transformer. A later model of the lamp is more simple.

color differentiation and contrast with certain classes of objects — as for instance blood preparations and some kinds of bacterial staining — are absolutely barred by the impossibility of focussing and the great length of exposure, during which also troubles from vibrations in the apparatus are greatly increased.

The housings of these three lamps, as well as of a new type of four and a half ampere arc lamp, are interchangeable on the illuminating unit shown in Fig. 79, in which an auxiliary condensing lens highly corrected for spherical aberration is also mounted.

The Vertical Camera. — The vertical camera is far more practically useful in low-power work than the horizontal, as it is only with the vertical camera that most objects can be photographed in the fresh state, i.e., without making permanent mounts. Very many objects would suffer such changes in the processes of preparation and mounting that photographs of them would be worthless.

Fig. 60 shows a simple support for any plate camera. The microscope stands on a low bench so that the ground glass can be inspected with comfort. The support carrying the camera is merely set over the microscope and may be removed at will. With the camera support removed, focus the object under the microscope, and see that the lighting is perfectly even all over the field. Use the plane side of the mirror. Then, taking care not to disturb the adjustments, place the camera support in position, wind the junction of the two tubes with a black cloth, focus sharply with the fine adjustment on the ground glass, and all is ready.

Fig. 61 shows an adaptation that allows the vertical camera to be used with any light and with any of the combinations of apparatus that have been described for the horizontal camera. A shelf, *S*, is arranged at one end of the

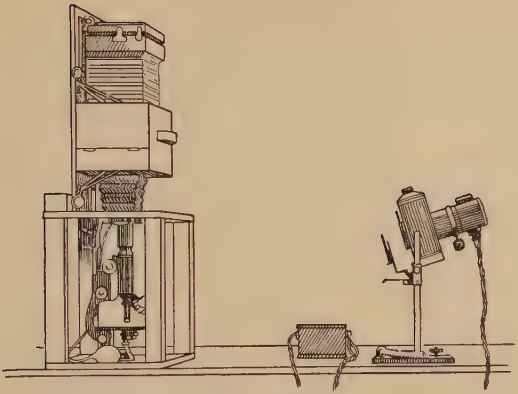


FIG. 60. — VERTICAL CAMERA.
With Bausch and Lomb illuminator, usual arrangement.

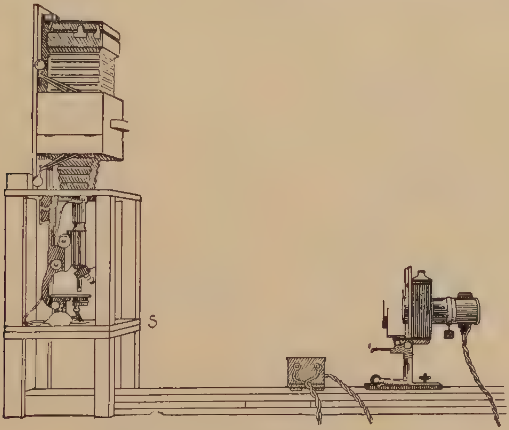


FIG. 61. — VERTICAL CAMERA.
Arranged with optical bed.

optical bed at such a height that, when the microscope stands vertically on it, the centre of the mirror is on the optical axis as arranged for the horizontal camera. As before, the object is focussed before the camera is put in position. Fig. 62 shows the arrangement for parallel light with a kerosene lamp.

It is rather easier to use parallel light with the vertical camera, since the microscope must be placed low to give access for inspection of the ground glass, and it is somewhat awkward and troublesome to focus the image of the light source on the diaphragm of the Abbe condenser, as must be done if convergent light is employed. However, convergent light may be used, as in any of the arrangements for the horizontal camera.

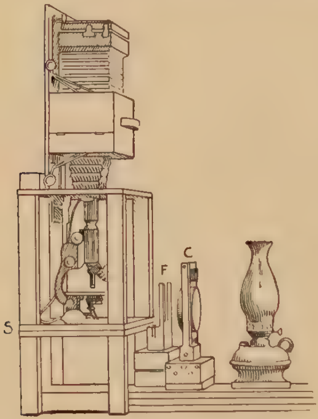


Fig. 62. — Vertical Camera with Kerosene Lamp.

The plano-convex lens, *C*, is about eight inches from the mirror. The lamp flame is at the principal focus of *C*. *F* is a support for a ray filter.

It is best to make for this arrangement a special optical bed about three feet long, so that it will take any of the combinations of apparatus, and attach the shelf support securely at one end. Any piece of apparatus may be transferred from one optical bed to the other.

With any of these arrangements the principal difficulty arises from the manipulation of the mirror. It requires some practice to secure proper illumination, as it is not easy to judge by the eye when the field is lighted with perfect evenness and with the greatest possible brilliancy. This adjustment must be made for each separate exposure. Without proper lighting, the exposures will vary or the photograph will be uneven. To secure even lighting remove the eye-piece and substitute for it the pinhole eye-piece. Manipulate the mirror until the image of the flame is seen to be at the exact centre of the back lens of the objective.

An arrangement that does away entirely with this troublesome manipulation of the mirror is shown in Figs. 63, 64 and 65. In fact it is the ideal arrangement for low and medium-power work if a lamp that permits of use in this way is available, as the apparatus is equally efficient as a vertical or a horizontal camera.

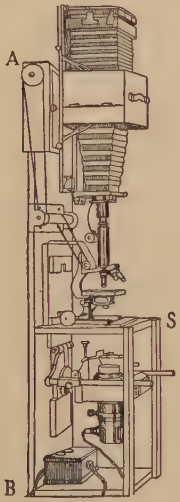


FIG. 63. — IDEAL ARRANGEMENT FOR LOW AND MEDIUM-POWER WORK.

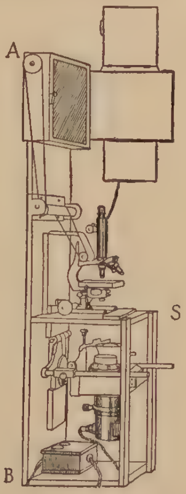


FIG. 64. — SAME WITH CAMERA TURNED TO ONE SIDE TO PERMIT THE ROUGH FOCUSSING

In Figs. 63 and 64, *AB* is a board four and a half feet long, near the lower end of which the lamp is securely held in position by cleats. A shelf, *S*, with a hole to permit the light to pass, is placed about eighteen inches from the floor. On this shelf the microscope stands, the mirror being swung to one side, and axial light direct from the lamp is used. When the light has once been properly centred and focussed, it requires no further attention. The illumination is constant and the exposures are uniform and even.

Any type and any length of camera may be used. The type shown in the illustrations is suitable for low-power work, but for the high powers one of more solid construction should be used. See page 221.

In shifting from the vertical to the horizontal position, the only change necessary, aside from the placing of the microscope, is the adjustment of the focus of the auxiliary condenser when convergent light is used. Parallel light is of sufficient intensity for most low-power work.

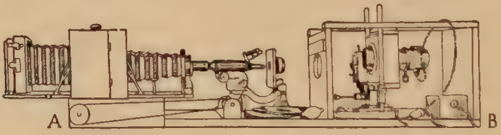


Fig. 65. — The Same Apparatus Used in the Horizontal Position.

Attention is called to the fact that the distance from the auxiliary condenser to the Abbe condenser is only thirteen inches. It is quite possible to photograph some types of objects at this distance with the oil-immersion objective, and, unless one has a lamp of higher intensity than the 24-watt Mazda, they must be photographed at this distance or they cannot be photographed at all with the ray filters necessary for proper contrasts. With objects of very delicate structure, however, diffraction fringes are likely to show around the edges. With coarser objects quite satisfactory photographs can be made.

The distance of thirteen inches is entirely correct with all dry objectives on account of their low numerical aperture, and because the numerical aperture of the sub-stage condenser must be cut down — as is usually done by partially closing the iris diaphragm — to avoid flooding the object with too much light.

By a little modification this arrangement may be adapted for any high-power work with the horizontal camera. Make the board, *AB*, about six feet long. Arrange the microscope support shown in Fig. 65 to slide on a guide strip. The camera and microscope may then be placed as shown in Fig. 65 or both may be moved to the left until the Abbe condenser is between thirty and forty inches from the illuminating unit.

Measurement of Magnification. — After the exposure of the plate, remove the object from the stage of the microscope without disturbing any of the adjustments, and put in its place a stage micrometer. With a pair of dividers obtain the length of the image on the ground glass plate of the camera of any number of divisions of the micrometer graduation. Apply the dividers to an accurate rule to find the exact length of the image. Divide this length by the known value of the divisions of the micrometer scale. For example, suppose a stage micrometer with a scale divided to hundredths of a millimetre is used, and the image of 10 of these divisions, as measured by the dividers and rule, is 7.5 mm. Then 7.5 mm., or 750 hundredths of a millimetre, divided by 10 hundredths of a millimetre gives 75 as the amount of magnification. This method gives a fairly accurate result.

If a stage micrometer is not available, the magnification may be roughly calculated, when the objective is the only lens used, on the geometrical principle of similar triangles. The length of the object and the length of its image are the bases of two similar triangles of which the focal length of the objective is the altitude of one, and the distance of the ground glass from the optical centre of the objective is the altitude of the other. The bases are to each other as the altitudes. The focal length of the objective is known, and the distance of the ground glass from the optical centre of the objective can be measured more or less accurately. We have then the ratio of the altitudes, and this is the same as the ratio of the bases. Suppose, for example, that the objective has a focal length of one-fifth of an inch, and the measured distance of the ground glass from the optical centre is thirty inches. Then $30 \div \frac{1}{5} = 150$, the magnification of the image. The results of this method are only approximate, owing to the difficulty of making an accurate measurement of the distance of the ground glass from the optical centre of the objective.

High-Power Work. — Focussing with the low powers presents very few difficulties. The specimens are comparatively coarse, the light abundant, and it is easy to determine when the focus is sharp. With the high powers it is quite different. Here the specimens are delicate and a very slight departure from accurate focus absolutely spoils the result.

It helps materially to cement with Canada balsam a cover-glass to the centre of the ground side of the ground glass, thus providing a clear area. A cross should first be marked with a lead pencil at the centre of the ground surface of the glass. The image may then be viewed on this clear space with a focussing glass and compared with the image as seen on the ground surface. The image of the cross and the image of the object should be in focus at the same time. Some workers dispense with the ground glass altogether, using a piece of clear glass simply as a support for the focussing glass. This method is too difficult for beginners and is also very trying to the eyes.

Three difficulties present themselves in high-power work, no one of which gives any special trouble in low-power photography. The first is caused by the shifting of the focus during exposure due to vibrations in the apparatus. To avoid these vibrations as much as possible, the camera should be of heavy and rigid construction. Both the back and the front should be firm and steady, especially the front, and the shutter, if one is used, should be of such construction that it can be operated with the least possible vibration. Either of the following methods is to be preferred to shutter exposure. With the tungsten lamps the exposures are very conveniently made by switching the light on and off, the room in the meantime being only dimly lighted, care being taken that no extraneous light falls on the microscope. The method of merely interposing a black card to cut off the light just below the Abbe condenser is the best one with all other sources of light.

An ordinary plate camera, while suitable for low-power photomicrography, is not at all adapted for work with the oil-immersion objective. A camera constructed like the one shown in Fig. 59 is of the correct type, or a simple box camera may be used. Some photographers, who are obliged to work under particularly trying conditions, suspend the whole apparatus from the ceiling by means of stout spiral springs and cords, or arrange springs, automobile tires laid flat, large corks or blocks of rubber under the base board or under the legs of the table supporting the apparatus. Every means to prevent vibrations must be adopted, or many disappointments will be met.

The intensity of the light used is also a factor of great importance. The longer the time of exposure necessary, the greater the liability of troublesome vibrations. Electric lamps have now almost universally superseded all other light sources for practical photomicrographic work. Each type has its own shortcomings. The arc lamp gives the advantage of shortness of exposure, but troubles arise, even when the direct current is employed, by the shifting of the spot of greatest intensity. The longer exposure required by the tungsarc lamp is at least partially compensated for by its absolute steadiness. The 24-watt tungsten lamp will serve the amateur while learning the first principles of high-power work, but the range of subjects that can be exposed to this light is greatly restricted, as already stated. If one is obliged to work with a light of low intensity, necessitating long exposures, his difficulties are greatly increased.

The second difficulty is caused by diffraction fringes about the edges of the object. Diffraction fringes may be due to either of two reasons. First, lack of sharpness in focussing; second, from closing down the iris diaphragm of the Abbe condenser too far. With low-power objectives of low numerical aperture, the iris should be closed sufficiently to fill the back lens of the objective about two thirds full of light, as may be seen by removing the eye-piece and looking into the tube of the microscope. With the iris in this position the image should be at its sharpest and the illumination at its best. Usually with the oil-immersion objective, which has a large numerical aperture, the iris must be opened to at least two thirds the diameter of the Abbe condenser, care being taken that there is no falling off in the sharpness of the image. Attention to this and to delicacy in focussing will do away with diffraction fringes and will reduce to a minimum the defects caused by specks of dust on the lenses. In low-power work the proper focus of the Abbe condenser will be found by moving the Abbe back from the object until the circle of light on the focussing screen is perfect and the light is at its greatest brilliancy. In high-power work the proper focus will be found when the Abbe is moved up almost into contact with the slide and the brightest illumination is secured. The best effects in *critical* work are produced by using immersion oil to connect the Abbe with the under surface of the slide. The focussing of the Abbe should be made, in either case, with the camera removed and the paper focussing screen set up in its place. *This is a matter of supreme importance.* The proper focussing of the Abbe condenser is quite as important as the focussing of the objective. Study carefully the sharpness of the image as the Abbe is moved towards and away from the object. Also notice the effects on the brightness of the illumination. When the image is sharpest, the illumination is brightest. Departure from the correct position of the Abbe changes the time of exposure enormously when a high-power objective is used. Careful study of these effects will be amply rewarded. See Chapter XVI.

The third difficulty is due to blotches and concentric rings produced by particles of dirt on the lenses, — particularly on the field-lens of the eye-piece. All the lenses should be kept as clean as possible, and special attention should be given to the eye-piece lenses. It is extremely difficult to get rid of every particle of dust. A piece of very old, much washed linen or very fine and soft silk should be used. A small stick of soft pine covered with the linen or silk will reach all parts of the lenses. Particles of dust that are not noticed at all in visual work become painfully evident in a high-power photomicrograph. Do not breathe on the lenses. Epithelial cells from the mouth are liable to be deposited on them, thus making a bad matter worse. The lenses may be washed with clear water. Xylol (Xylene) or benzole may also be used.

Many eye-pieces are faulty. A practically perfect eye-piece is necessary for good work with high-power objectives. In low-power work little trouble is experienced from the defects. In visual work they are avoided by revolving the eye-piece, and one scarcely realizes to what extent they exist. In high-power photomicrography such defects spoil the results. If all attempts to clean the eye-piece fail to overcome the difficulty, order of the makers an eye-piece especially selected for photomicrographic work. With achromatic objectives the Huyghenian type, if perfect, is entirely suitable. With apochromatic objectives the compensating or projection type should be used.

To locate specks of dust or defects in the eye-piece focus sharply on a suitable object with the oil-immersion objective. Revolve the eye-piece. Any specks or defects will revolve. To determine whether they are on the eye-lens or field-lens, loosen the eye-lens and, holding the eye-piece as far down in the tube as possible, revolve the eye-lens and the field-lens separately. Specks or defects are thus easily located.

Dirt on the back lens of the objective may be detected by looking at the eye-point just above the eye-lens of the eye-piece with a magnifying glass. When the magnifying glass is held in the right position, some inches above the eye-piece, it focusses on the so-called Ramsden circle, which is simply the aerial image of the back lens of the objective formed by the lenses of the eye-piece. Make the examination with the iris diaphragm of the sub-stage condenser set at different openings so that the back lens of the objective is filled from one half to three quarters full of light. This examination also detects imperfections in the objective, dirt on the front lens, or air-bubbles in the immersion oil. While examining the eye-piece or objective, the microscope should be arranged as for visual work. No attempt should be made to look through the microscope when arranged for photomicrography with an intense light unless a colored glass or one of the dense ray filters is interposed to moderate the light and guard against injury to the eyes.

Photography. — It is to be supposed that one who wishes to engage in taking photographs through the microscope has already had some experience in general photography. If not, it is desirable to practice making exposures out of doors and afterwards indoors until some ideas are gained as to the light values as seen in images on the ground glass and some judgment is attained as to the time to be given in exposure. This experience should be acquired by the use of a plate camera. Exposing for snapshots has no value for this purpose. Exposing indoors gives especially valuable information. It is still more important to acquire experience in the development of dry plates and in printing the photographs. To this end select plates and printing papers of any reliable manufacturer and follow the directions given for exposure, developing and printing.

While such general experience is desirable, it is by no means essential. The first experience may be secured by practice with the simple arrangements of the photomicrographic camera shown in Figs. 50, 51, 62 or 63, using a low-power objective, say the 16 mm.

The Dry Plate. — White light is made up of an infinite number of rays of different lengths. When these pass through a prism, they are differently refracted. When the light passes through an uncorrected lens, the same uneven refraction occurs, so that a more or less perfect spectrum is formed around the edges of the image. Achromatic lenses are corrected to cause the rays producing yellow and green — the two colors most active visually — to come to a focus as nearly at one point as possible, thus doing away in large measure with the blurring effects caused by rays focussing at varying distances from the lens. Apochromatic lenses bring the rays of three colors as near to one point as possible. The apochromatic lens is therefore much to be preferred in photographic work. Panchromatic dry plates have been treated chemically to make them theoretically sensitive to all the colors of the spectrum. Work of the highest perfection with certain types of objects can only be done with panchromatic plates and apochromatic objectives used with highly corrected sub-stage and auxiliary condensers and with ray filters that give approximately monochromatic light. With other large classes of objects, a less perfect equipment suffices for very satisfactory work. Achromatic objectives with or without the ordinary Abbe condenser, and with no ray filter for magnifications below seventy-five or one hundred diameters, give good results with some objects on ordinary plates, and with orthochromatic plates and yellow or green filters with the highest magnifications, — results that can be distinguished from those obtained with the most costly equipments only by experts.

It is assumed that most workers for whom these pages are intended will use ordinary achromatic objectives. It is desirable then to find some means of counteracting the defects of these lenses. This is done by the use of orthochromatic or panchromatic plates and ray filters. The orthochromatic plate has been treated chemically to make it more sensitive to yellow and, to a less extent, to green, — the rays with which the achromatic lens works best. To get rid of at least a portion of the violet and red rays, which would blurr the image when high powers are employed, a ray filter, or color screen, is used; these will be considered later. Since the orthochromatic plate is more sensitive to yellow and green than the ordinary plate, it will not bear exposure in the dark room to the extent that an ordinary plate will. Care must be taken to see that the dark room light is *safe* for this plate. Ordinarily a *good* ruby glass and one thickness of orange *post-office* paper give sufficient protection, unless the light is of too high power. Before beginning actual work, test the light in the usual way by withdrawing the slide half way from over a plate, expose for ten minutes in the position where the developing tray is to be used, develop and fix the plate. If any difference can be seen in the two halves of the plate, the light is not safe.

The latest improvements in orthochromatic plates have greatly increased the latitude in exposure and have rendered it much easier to obtain sufficient contrast to bring out the details of the object and to insure a clear white background. The double coated orthochromatic plates are claimed to be superior to the ordinary orthochromatic plates in these respects. They are good plates, but a somewhat thorough trial of one of them has failed to produce results superior to those obtained on single coated orthochromatic plates.

To secure the highest degree of color differentiation, a panchromatic plate must be used. These plates possess the advantage over orthochromatic plates that a ray filter of any color may be used, i.e., monochromatic light of any of the seven colors of the spectrum may be employed. Various panchromatic plates are on the market, and doubtless all possess good qualities. The Wratten "M" plate is the result of much investigation for photomicrographic work, first in England, afterwards in this country. These plates are *backed*, which affords the best possible protection against halation troubles. They also give strong contrasts, which is important in securing detail and at the same time a clear white background. The photographs made with them are much harsher than those made with orthochromatic plates. Panchromatic plates *may* be used with all classes of objects. They *must* be used with objects requiring a red ray filter. For nearly all other classes of objects the orthochromatic plates are to be preferred, and all the ray filters except the red may be used with them.

No better advice can be given to a beginner than to select an orthochromatic plate at the start and to use no other until considerable experience has been gained. If the Seed L Ortho. plate is selected, advantage may be taken of the exposure factors for those of the Wratten filters which can be used singly or in pairs with this plate. If the Cramer Medium Iso. or Instantaneous Iso. or the Standard Orthonon (double coated) plate is selected and the Cramer ray filters are used with the arc light, the exposure factors for these plates and ray filters are available. See page 239.

It is well for the beginner not to change for a time the distance of the ground glass from the microscope, as the time of exposure changes for each change in the distance. Rather select such microscopic specimens as can well be taken at the distance adopted and practice with these until familiarity in handling them has been acquired. The same may be said of changes in objectives, eye-pieces and ray filters. Practice with one combination, beginning with a low power, until the general principles have been learned. Sections of plant stems or of animal tissues that show definite structure under a 48 mm., 36 mm. or 16 mm. objective and low-power eye-piece are especially suitable at first. When experience has been gained, other combinations of apparatus, other plates and ray filters may be used.

The effects in the photomicrograph vary much with different plates. In fact these variations depend almost as much on the qualities of the plate as on the ray filter used. When a thorough familiarity with the principles has been gained by the use of some one plate, it is desirable to know by experience the effects that can be produced with different plates.

Ray Filters. — It is to be remembered that the yellow and green rays for which the achromatic objective is corrected are not the most active photographically, that the most actinic rays do not focus at the same point with them, and the image is blurred if the magnification is high. To prevent this blurring of the image, it is desirable to get rid of the rays that cause it. Pieces of suitably colored glass or gelatine or chemical solutions of the proper color interposed in the path of the light accomplish this. Anything thus used is called a *ray filter* or *color screen.*

A second reason for the use of the ray filter and orthochromatic or panchromatic plates is that in photographing objects showing different colors — as in the case of double or multiple stained objects — a light as nearly monochromatic as possible usually best brings out the proper contrasts of the different parts. Some specimens will take well, as far as the color values are concerned, on any plate and without a ray filter if the magnification is low, but many can be satisfactorily photographed only on orthochromatic or panchromatic plates and with a filter. With high magnifications a filter should always be used.

The Wratten " M " filters are gelatine films that have been stained with dyes chosen so that the nine filters give theoretically the whole range of the spectrum. By combining these filters in pairs, practically monochromatic light of any color can be obtained. The Cramer ray filters are also gelatine films stained with dyes. Either the Wratten or the Cramer filters are all that any ordinary worker will need. A light yellow filter should be used by the beginner.

Liquid ray filters may be made in great variety by dissolving different dyes and chemicals in water. A suitable cell to hold the solution is easily improvised. Clean two spoiled negatives. To one of these cement with shellac cement a rubber ring such as is used on glass preserve jars or one cut from rubber of any desired thickness. Put a heavy weight on this until the shellac is thoroughly dry. Then laying the plate on a level surface fill the ring with the solution. Place the second glass plate over the ring, but not cementing it, and clamp the two plates together with strong letter clips, taking care that the clips bear evenly directly over the rubber ring so as to prevent uneven strain on the glass. As such a cell is air tight, there is no evaporation of the liquid, and the principal objection to liquid filters is avoided. A liquid filter thus constructed is practically as convenient to use as a gelatine film filter.

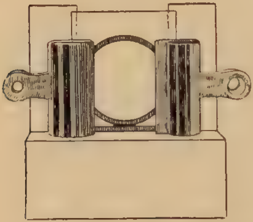


FIG. 66. — CELL FOR LIQUID RAY FILTER × $\frac{1}{4}$.

The two uprights at the back take a gelatine film filter.

For a pale yellow liquid filter, use a saturated solution of picric acid. This filter absorbs the most of the violet light and transmits the light through the remainder of the spectrum. Such a filter is of the type adapted for general use with orthochromatic plates and is suitable for the beginner to use at the start. A saturated solution of dichromate of potash makes a good orange-yellow filter. It absorbs the violet, indigo, blue, and part of the blue-green, and transmits the rest of the spectrum. A good yellow-green filter is made by mixing in varying proportions saturated solutions of acetate of copper and bichromate of potash. A little strong acetic acid must be added to each solution before mixing to prevent the formation of a precipitate. This filter absorbs the violet, indigo, blue, part of the blue-green and the greater part of the red; it transmits part of the blue-green, all of the green, yellow and orange, and some of the red; it is well adapted to correct the deficiencies of achromatic objectives when panchromatic plates are used. For a blue filter add ammonia to a saturated solution of copper sulphate until the precipitate that forms is dissolved, and a clear blue solution is obtained. This filter transmits the violet, indigo and part of the blue and absorbs all the rest of the spectrum. For a red filter use a 1:1000 solution of aniline red. This filter absorbs from the violet end of the spectrum through the yellow; it transmits the orange and red. Almost any other variety of colors may be had by using 1:1000 solutions of other aniline dyes. This equipment of filters will serve the amateur well.

The statements as regards the absorptions and transmissions of these filters are only generally true as are similar statements about any filters. No filters perfectly accomplish what they are intended to do. All pass some light in the absorption areas and cut out some light from the transmission areas. A spectroscope only will determine. The absorptions and transmissions also vary with any filters as to whether the light of the sun, lime light, mercury vapor, electric arc, incandescent electric, gas, acetylene or oil is used.

The thickness of the liquid filter or strength of the solution will also depend on the intensity of the light employed. With a light of high intensity the solutions may be used in full strength in cells several millimetres thick. With lights of low intensity a cell two millimetres thick is sufficient.

The liquid filters are excellent, and the cost is merely nominal. Since the solutions may be made up fresh with very little trouble and since the gelatine filters fade after a time, the liquid filters possess the advantage of reliability of color over the gelatine films, with the exception of those made with the aniline dyes, which are in themselves variable and unstable. They are also more transparent. Consequently focussing is easier and the time of exposure less, — advantages of considerable importance in some cases.

The scientific way to use ray filters is to examine the light transmitted by the filters and also by the stains with a pocket spectroscope and select the filter whose transmission is most likely to meet the requirements of the particular case. The visual appearance of the color of a filter or of a specimen does not always, in fact not usually, indicate what colors are absorbed and what are transmitted. This is especially true of the aniline dyes. The only way to determine accurately is by the use of the spectroscope. Not many of those for whom these pages are intended will own this instrument or care to give the subject the attention necessary to obtain reliable information. Most workers will depend on their own experiments or on the results worked out by others. For this reason the Wratten and Cramer filters appeal, as very valuable data have been worked out for them.

To secure the maximum of sharpness of definition and detail with an achromatic objective, the yellow and green filters and an orthochromatic or panchromatic plate should be used. It is, however, sometimes necessary to sacrifice in these particulars to secure proper contrast with the background or between the parts of the object.

It will give a better insight into the principle on which ray filters work in producing contrast, if we consider the photographing without a filter of an object with opaque areas, for instance the foot of a fly that has not been rendered translucent by treatment in the preparation for mounting. The dense opaque portions cut off all light, the parts of the plate covered by them are not acted on at all, and when the plate is developed are perfectly transparent; the less dense parts transmit some light, and the parts of the plate covered by these are acted on in proportion to the amounts transmitted; the parts of the plate not covered by the image of the object are acted on more fully and, if the exposure and development have been correct, will be dense and dark in the negative. When a print is made, the transparent parts of the negative give a dense black with no detail, the intermediate parts show gradations of black and white giving the details, the dense background cuts off all light and shows clear white in the photograph. Any opaque object provides the conditions for maximum contrast. The gradations of contrast in a translucent object are in proportion to the amounts of light transmitted. The ray filter regulates the amount and the character of the light. If, for instance, we photograph an object of uniform color with a ray filter whose color is exactly complementary to that of the object, we cut off all light, have the condition of maximum contrast, and the object photographs as an opaque object would. By adjusting the color of the filter to the different colors in an object to allow light to pass in different degrees through its different parts, we have gradations, and the details of structure show in pleasing contrast with one another and with the background. The opacity and the colors of the object are just as important as the color of the filter. On the correct adjustment of the color of the filter to the colors of the object depends the quality of the photograph. A ray filter that cuts off the light transmitted by the object, i.e., one of complementary color, gives maximum contrast and minimum detail; one that transmits light of the same color, i.e., a filter of the same color, evens up the light transmitted by the different parts of the object and so gives minimum contrast and maximum detail.

The narrower the bands of transmission of the filters, the more perfect is the control of the light and in difficult cases the more perfect the result. The Wratten or Cramer filters combined in pairs give moderately narrow transmission bands in any part of the spectrum. Liquid filters of suitable colors properly combined may be made to accomplish the same end, but a spectroscope is absolutely indispensable in selecting the colors. For most amateur work the transmissions of the Wratten or Cramer filters used singly or of the liquid filters already described are all that are necessary. In fact in many cases better photographs are made with the broader bands of light.

Objects colored pure red, brown, yellow or green photograph on an ordinary plate as opaque objects, since this plate is not acted on by these colors and all other colors have been absorbed by the object. Only specimens of extremely delicate and open structure give good photographs. On an orthochromatic plate without a filter, yellow and green objects give better results than on an ordinary plate, since the orthochromatic plate is sensitive to these colors, but show more uneven contrasts than when a yellow or green filter is used. Red objects can be properly photographed only on panchromatic plates, which are sensitive to this color, and without a filter these plates give better results than the ordinary or orthochromatic plates; with a filter the contrast can be more fully regulated.

The maximum or minimum contrast does not usually give the most satisfactory results as regards the details and relative contrasts. To understand this better, it is well to consider particular cases.

Suppose that an object stained orange or red is to be photographed. If an ordinary plate and no filter are used, the photograph will be taken by the blue, violet and ultraviolet rays which are the only ones that greatly act on an ordinary plate. With these rays the orange or red specimen takes as black, the contrast is too great, the details are obscured, and the result is not satisfactory unless the structure is delicate. If a blue filter and an orthochromatic or panchromatic plate are used, the result is similar. If an orange or red filter and a panchromatic plate are used, the contrast will be very slight, the photograph will be flat, characterless and useless, if its structure is delicate. If, on the other hand, filters intermediate between the blue and the orange — green or yellow or a combination of these giving yellowish-green — and a panchromatic or orthochromatic plate are used, the contrast and details in the photograph will be intermediate between the extremes. Trial only will determine which filter and which plate is best for a particular specimen. The example taken is extreme. In most cases we have to deal not with a uniform color in the specimen but with a combination of colors, either those inherent in the object itself or those obtained by multiple staining or by the selective action of the object on a single stain; we are also in many cases dealing with areas that have taken the stain very faintly or not at all, and usually these offer the greatest difficulties to bring out the details and secure the proper contrast with the background. It is impossible in some cases to secure a satisfactory photograph of a poorly stained delicate specimen. This is especially true if the magnification is high, as for instance with preparations of bacteria and blood; the only hope in such cases is to use a filter of the color complementary to the color of the object so that maximum contrast may be secured. On the other hand, if the specimen is coarse and deeply stained in one color, a good result can often be secured by using a filter of the same color to minimize the contrast and bring out the details.

Another illustration will perhaps make the matter clearer. Suppose a preparation of tubercle bacilli in sputum is to be photographed, the bacilli being stained a deep red with carbol-fuchsin and the sputum smear a delicate blue with methylene blue. If an ordinary plate and no filter or an orthochromatic or panchromatic plate with a blue filter is used, good contrast will be obtained for the bacilli and they should stand out well in the photograph but probably with some lack of sharpness of focus, if an ordinary achromatic objective is used, as the light is not suited to the objective. We shall have the minimum contrast for the blue portions of the specimen, and they, being delicate, will be almost or quite invisible in the photograph. If a red filter and a panchromatic plate are used, good contrast will be obtained for the blue portions of the preparation, but the bacilli will be very faint. Filters of the intermediate colors — green, yellow or orange — give varying emphasis on the two colors of the object. If a green and an orange filter are used together, giving a yellowish-green light, the contrast between the red bacilli and the blue background will be still further evened up, and, as the light is that best suited to the corrections of an achromatic objective, there should be absolute sharpness of focus and no diffraction bands, if the focussing has been properly done. There is some sacrifice of contrast with both the red and the blue, but the result is far more satisfactory than when extreme contrast for either is aimed at.

So with any other specimen. The aim should be to compromise the extreme contrasts of the different parts so that the general effect shall be the best. This is a general principle. *If the colors of the object are separated in the spectrum, a filter midway between will ordinarily give the most satisfactory photograph. If the two colors of the specimen lie next to each other in the spectrum, the filter should usually be of the color farthest to one side.* It must be remembered, though, that the visual colors in both filters and objects are not a true indication of the colors actually transmitted. This introduces an uncertainty which can only be cleared up by using the spectroscope or by experimenting with different filters. If we could have a series of filters giving absolutely monochromatic light of one wave length, the problem would be simplified, as we should then have only the uncertainty as to the transmissions of the colors of the object.

No such filters are known. The nearest approach to them at a reasonable cost are the Wratten and Cramer filters.

A few general suggestions, the results of the experience of many different workers, may be added as to the use of filters with various common stains and preparations.

The most generally useful filter is a yellow or yellow-green. With such a filter a very large number of objects can be successfully photographed.

To secure contrast with blue stains generally, use a red or violet-green filter with a panchromatic plate or a yellow, yellow-green, or orange-green filter with an orthochromatic plate. With yellow and brown stains the blue filters usually give maximum contrast, though ordinary plates and no filter give high contrast with yellow, brown and red. With red and purple stains the green filters are best; with green stains, the red and orange filters.

Experience has shown that with the haematoxylin stains a strong yellow-green filter usually secures the best results. With eosin and carmine stains, while a strong yellow or yellow-green serves well for brightly stained objects, delicate and faintly stained specimens require a pure green. For sections double stained with carmine and one of the aniline greens, a yellow, yellow-green or green filter gives good results. For sections double stained with eosin and haematoxylin, a yellow-green should be used. For Bismarck-brown use a blue filter. For gentian-violet use orange-green, but if the staining is dense the yellow filters work well. For blood preparations use an orange-green. For bacteria preparations in general a green, yellow or yellow-green filter may be used; the choice of the filter depends, of course, on the staining; for eosin and methylene blue use orange-green; for the Gram stain use a pure green. With yeasts, plant fibres, starches and fungi, green or yellow-green filters are generally advised. For algae and similar objects mounted fresh in a drop of water, the yellow and orange filters are good; if panchromatic plates are used the red are better. For insects and similar yellow or yellow-brown objects use a blue filter for contrast and a red for details. In the difficult work of photographing diatoms, the green filters have been found most useful, though many give good results with yellow filters. The blue and violet filters are used to increase the resolving power of objectives in photographing the very fine markings on diatoms and similar objects, but, of course, are not well adapted for use with achromatic objectives; the shorter the wave length of the light used, the greater is the resolving power of lenses. For metal surfaces photographed by reflected light, for chemical crystals and for rock sections use a pure green filter.

It is well to remember that some specimens photograph better with no filter at all, as the contrasts are better evened up by using all the colors of the light.

Some idea of the proper filter to be used can be gained by observing the image as seen on the ground glass or on a paper screen placed in the position of the ground glass, introducing one ray filter after another, noting which brings out the details of the object with greatest evenness and clearness or which emphasizes the particular detail that it is desired to show most clearly. This idea will, however, be only a general one. The only way to determine in some cases is actually to try taking the photograph.

The ray filter may be introduced anywhere between the light source and the ground glass of the camera, provided that all the light that reaches the photographic plate can pass through it. Just in front of the auxiliary condenser is the best position, especially if the filters are mounted between pieces of ordinary glass, as spherical and chromatic aberrations will here have the least effect. For very *critical* work, in order to avoid spherical and chromatic aberrations as much as possible, the filters should be mounted between *optical flats*, i.e., pieces of glass whose surfaces have been ground to optical planes, the two sides being exactly true and parallel. These are expensive and for most amateur work are not necessary.

When a ray filter is used, the time of exposure is, of course, greater than when photographing without one. To make practical use of a series of filters, it is necessary to know the approximate exposure factors, i.e., the time of exposure when each filter is used compared with the time for the same plate when no filter is used. These may be obtained for several light sources from Wratten's *Photomicrography*[1] for the Wratten plates and filters and for the Seed L. ortho. plate. If the Cramer plates and filters are used with the arc light, see the article by Professor Petrunkevitch of Yale University in the *Anatomical Record* for Nov. 20, 1920. If liquid filters are used, they must be determined by trial. This gives most valuable practice. A series of experiments to determine the exposure factors for a set of filters gives an understanding of the principles underlying the use of filters and a knowledge of exposure that volumes of description cannot furnish.

For further discussion of ray filters consult Barnard's *Practical Photomicrography* and Hind and Randles's *Handbook of Photomicrography.*

Exposure. — No fixed rules for the time of exposure can be laid down. The time varies with different types of objects with the same arrangement of apparatus, and for the same object for each change of objectives, eye-pieces, auxiliary condenser, camera extension or ray filter and, of course, for changes in the dry plates used. The qualities of the various components of the optical system used affect it. It varies with different sources of light. Changes in the distance of the light source from the microscope cause great variations. The character of and the proper focussing of the sub-stage condenser and the proper opening of its iris diaphragm are important. The time of exposure depends on so many varying conditions that any attempt to give the times for any specified arrangement is likely seriously to mislead. The beginner must learn by experience with his own apparatus and must accept the times given by any other worker as only approximate.

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[1] This is a little pamphlet published to advertise the Wratten plates and ray filters and can be obtained from the Eastman Kodak Co.

In practice exposure, the time at each successive attempt should be *doubled* rather than increased by small increments. The common method used in general photography of making several trial exposures on the same plate tends to economy. See Wratten's *Photomicrography*, page 30.

The time should be estimated as closely as the worker's experience permits and a trial plate exposed, giving the first third of the plate one half of that time, the second third the time estimated, and the last third double the estimated time. It should also be borne in mind in using exposure factors for any filters and plates that these factors are only approximate and also vary much with the character of the object being photographed. A trial plate should therefore be exposed for one half the time, the full time, and double the time reckoned by the exposure factors. The beginner, using an orthochromatic plate and a dark room light that is safe for the plate, should watch the development of the trial plate for fifteen to twenty seconds, noticing on what part of the plate the image *just begins to show* in that time. The tray is then covered and the development continued for the full time required by the developer used. The time given to that part of the plate on which the image *just begins to appear* in from fifteen to twenty seconds is the correct time of exposure for the object. The same time of exposure will be found correct for other objects of the same type, but if an object is different in character, especially in staining or thickness, a new fractional trial exposure may have to be made for it. In this first practice exposure it is best to use an object that will leave portions of the entire length of the plate without an image, the image of the object also appearing along the entire length of the plate. With such an object not only the appearance of the image but also the appearance of the uncovered portion of the plate can be better judged.

Developing. — Individual preference will determine what developer to use. The pyro-soda developer is best for softness in the photograph and is excellent with certain classes of objects and certain plates. Many objects require sharpness of contrast rather than softness. For these hydroquinone, metolquinone or elonquinone is to be preferred. After mastery of the use of either type has been gained, a similar mastery of the other type is desirable. Experience will soon enable one to judge which type is to be preferred for a particular object or a particular plate, or if good results are not secured with one, the other may be tried.

If the time and temperature method of developing is employed, it is very important that a fresh portion of the developer be used for each plate. The pyro-soda developer oxidizes quickly, and a fresh portion should be used whatever method is employed. If the tray and inspection method is employed, the same portion of the hydroquinone developers may be used for three or four plates, but the time of development will be increased for each successive plate. If the time and temperature method is used, this lengthening of the time will vitiate the results.

With the hydroquinone developers better contrast and sharpness are obtained on some plates by using old developer diluted with an equal volume of water for half of the period of development; then pouring off this and replacing it with fresh developer for the remaining half of the period. With pyro-soda greater sharpness is secured by increasing the proportion of pyro, and greater contrast by increasing the sodium carbonate. Such changes affect the time of development and it requires experience to make use of modifications of the developer, which are especially difficult of application if the time and temperature method is employed. The beginner will therefore do better to confine himself to the use of normal developer until he has gained considerable experience.

There are four methods of development in common use. *First,* the old tray method in which the completion of development is judged by the inspection of the image as seen through the back of the plate. In the hands of an expert this method is undoubtedly the one that will give the best results with ordinary plates, but it is decidedly not the best method for the novice.

Second, the factorial method, by which the number of seconds that elapse from the instant the developer is poured over the plate to the first appearance of the image is multiplied by a number or factor, as it is called, previously determined for the developer used. The difficulty in the use of this method is to determine *exactly* the first appearance of the image, for an error in this will be large when multiplied by the factorial number.

Third, the time and temperature method. By this method the time must be determined at a given temperature for the development that will give satisfactory results. It is then necessary merely to see that the developer is at the required temperature and allow the development to proceed for the required time. This is without question the best method for the beginner. With panchromatic plates it *must* be used, and it is almost equally necessary for orthochromatic plates, for a light sufficiently strong to show the delicate structures of a microscopic image will almost inevitably fog the plate.

The plate holder is loaded in absolute darkness. When ready to develop, have the tray and already mixed developer in convenient positions. The room still being lighted by an *ordinary* electric light, note the time, switch off the light, take the plate from the holder and pour the developer over it in absolute darkness, rocking the tray. If now the plate is orthochromatic, switch on the *dark room* light and watch to see if the image faintly appears within fifteen to twenty seconds. It is easy to learn to count the seconds with sufficient accuracy. The counting should begin the instant the developer is poured over the plate. Switch off the *dark room light.* A light-tight cover — a pasteboard box cover is all that is needed — is then placed over the tray and an *ordinary* electric light switched on until the time of development is complete. The light is then switched off while the plate is washed, transferred to the fixing bath and again covered. The light may then be switched on again.

When one has become thoroughly familiar with this method while using orthochromatic plates, the transition to panchromatic plates becomes easy, and the difficulties anticipated in developing without a dark room light disappear. The plate is developed without inspection, and the result is determined after the negative has been completed. No special clock is necessary. The keeping of the time is very simple, as the room is fully lighted when the times of beginning and closing of the development are observed. It is merely necessary to estimate the few seconds required to remove the plate from the plate holder, place it in the developer and cover it. The secret of the method is to weigh the chemicals accurately and measure accurately the water in which they are dissolved, so that there may be no variation in the strength of the developer. Then, if the temperature is accurately maintained, the results will be very uniform, — probably much more uniform than a novice can secure by the tray and inspection method.

This method is especially desirable when working with developers which contain metol or other substances which poison or color the skin, as the hands are brought in contact with the developer only for the purpose of removing the plate for washing. Another advantage is that it reduces the strain on the eyes to a minimum.

Fourth, tank development. This is simply an application of the time and temperature development. In recent years this method has come into common use for both films and plates. It is the method to use when a large number of plates are to be developed at a time. Details of the method are given by the makers of dry plates.

It is to be remembered that the time of development necessary to produce photomicrographic negatives with good printing qualities to bring out details and at the same time give clear and brilliant whites in the background is considerably more than that necessary for a good landscape photograph.

Whatever method is used, careful attention to the temperature is of prime importance. Temperatures between 60° and 70° F. are most suitable. The ideal way would be to have the dark room at the correct temperature and to let the chemical solutions and developing trays stand until they have acquired this temperature. As a constant temperature room is usually not available, it is well to follow the suggestion made by one of the makers of dry plates, to place the developing tray in a larger dish containing water, maintaining the correct temperature by adding hot water in winter and ice in summer. A thermometer should of course be used.

The writer finds that the following modification of the Eastman formula gives good results. The increase in the amount of sodium carbonate is to give greater density and better printing qualities to the negative.

Pyro-soda Developer.

A

Water	16	oz.	or 500 c.c.
Sodium or potassium meta-bisulphite	90	grains	" 6 gms.
Pyro	1	oz.	" 28 gms.

B

Water	16	oz.	or 500 c.c.
Sulphite of soda (dry)	1.5	oz.	" 45 gms.

C

Water	16	oz.	or 500 c.c.
Carbonate of soda (dry)	1.6	oz.	" 48 gms.

For use take A = 1 part; B = 1 part; C = 1 part; water = 7 parts.

Development for 7 minutes at 67° F. gives strong contrast.

Petrunkevitch in the *Anatomical Record* for Nov. 20, 1920, highly recommends:

Pyro-acetone Developer.

A

Water	500 c.c.
Oxalic acid	1 gm.
Pyro	30 gms.

B

Water	1000 c.c.
Sulphite of soda (dry)	120 gms.

For use take

Water	120 c.c.
A	15 c.c.
B	30 c.c.
Acetone	6 c.c.

Develop 6 minutes at 65° F.

The Cramer Dry Plate Company recommend:

Hydroquinone Developer.

A

Water	32	oz.
Hydroquinone	1.5	oz.
Sulphite of soda (dry)	1	oz.
Sulphuric acid	60	minims.

B

Water	32	oz.
Carbonate of soda (dry)	1	oz.
Carbonate of potassium	3	oz.
Bromide of potassium	120	grains
Sulphite of soda (dry)	3	oz.

For use take equal parts of A and B.

Develop from 4 to 8 minutes at 70° F. according to the density desired.

The Eastman "M.Q." (elon-hydroquinone) developer gives good results. It is especially convenient when developing is to be done only occasionally. The time is about the same as for the Cramer hydroquinone developer. With many classes of objects the best results are secured by adding one or two drops of a saturated solution of bromide of potassium to each ounce of the developer and continuing the development for 9 minutes at 67° F.

The conditions under which the negative is dried after development are also important, but cannot always be easily controlled. A rather high temperature with a current of dry air affords the best conditions. Some makes of plates increase in density during drying; others lose in density. The amount of this increase or decrease varies with the time of drying and is a source of great vexation while one is acquiring familiarity with different makes of plates.

Printing. — A paper with a hard, smooth, glossy surface should be selected, and a process that produces sharp contrasts between deep blacks and clear whites should be employed. The directions that come with the different makes of paper must be the guide, or experience with different papers and processes must determine what will best suit each individual worker. A so-called developing out paper, such as regular glossy velox, is most convenient. The processes are much simpler and more uniform than those of sun-printing, and possess the advantage that one is absolutely independent of sunshine and may do the work in the evening. For some purposes a sun-printing paper like solio is desirable.

Lantern Slides. — Lantern slides may be made by contact from the negatives of photomicrographs in the same way as from ordinary negatives. The process is precisely the same as the process of making a gaslight print, and is very simple. Special, slow dry plates, or positive plates, are made for the purpose. In the dark room place one of these over a negative in the printing-frame so that the films are in contact. The exposure is made by lamplight, and must be learned by practice. It varies from five to thirty seconds, according to the density of the negative and the character of the light used. The lantern slide is developed in the same manner as a negative, using the formula for the developer that accompanies the plates. After development the lantern slide is washed free of the developer and fixed in sodium hyposulphite. The sodium hyposulphite must be thoroughly washed out. When the plate is dry, it is covered with a piece of thin, clear glass, an ornamental mat being placed between to cut out the peripheral portions, and the edges of the two pieces of glass are bound securely together by means of strips of black gummed paper.

Since negatives necessarily vary in density and since the lantern slide plate will bear exposure to dark room light, the tray and inspection method of development is to be preferred in this work. The development is much shorter than for photomicrographic negatives. The image should be carefully watched and as soon as the details are clear, the development should be stopped. A thinly printed lantern slide is much better than a dense one, provided all the structure of the object is shown.

Lantern Slide Diagrams. — Flow a lantern slide cover-glass with very dilute xylol balsam or with any good varnish made very thin with turpentine. Stand on edge until dry. Diagrams may be drawn on the varnished surface with a small ball-pointed pen and India ink. The prepared slide may be used like tracing paper to copy from books or to trace the microscopic image projected as for drawing.

Enlargements. — More satisfactory results are often obtained by photographing an object at a rather low magnification and then making enlargements on enamel bromide, carbon black, velox or cyko paper than by photographing at a high magnification. It is easy to make enlargements from good negatives. A simple, inexpensive and convenient arrangement of apparatus is as follows: Insert a condensing lens — one four inches in diameter is large enough for the $3\frac{1}{4}'' \times 4\frac{1}{4}''$ plate — in the partition between the dark room and an adjoining room. In the adjoining room place a 250- to 500-watt concentrated filament Mazda lamp so as to throw a strong converging light through the lens into the dark room. In the dark room enclose the light beam with a box-like arrangement open at the ends. Accurately centre a photographic lens on the end of a second box-like arrangement constructed to slide over the first one, allowing no light to pass except through the lens. With the sliding box removed place a negative in a suitable holder close to the condensing lens. Replace the sliding box and with it focus onto a screen set up at the proper distance to obtain the desired enlargement. When all is ready, shut off the light from the photographic lens and fasten the sensitized paper to the screen with thumb tacks. The exposure and development are no more difficult than in the making of prints direct from the negatives. Lantern slides may also be made in a similar way. The dark room may of course be lighted with an ordinary dark room light.

Some Troubles of the Photomicrographer. — One of the earliest difficulties of the beginning photomicrographer is unevenness in the print, i.e., parts of the print are too light, other parts are too dark. Parts show the structure of the object clearly, other parts are too dense, and the structure is clogged. This may be because the illumination is not uniform, which results from the fact that the various components of the optical system are not accurately centred on the optical axis of the microscope. The trouble may be with the light source, the auxiliary condenser or the sub-stage condenser. Each should be examined in turn. The centring of the light and auxiliary condenser, directions for which are given on pages 203 et seq., should be again adjusted. Directions for centring the sub-stage condenser are given on page 281. Another possible source is faulty development. The developer may not have been poured evenly at the start, and some portions of the plate were acted on more intensely than others. Another source of the trouble may be the fact that the object photographed was thicker or denser in some parts than in others, or that it was not mounted perfectly flat. No remedy can be found for this fault. Another source of the trouble may be that the camera back is not accurately adjusted at right angles with the optical axis. If an old mount is being photographed, the mounting medium may have discolored unevenly. A denser yellow ray filter will sometimes be the remedy. In low-power work, the lens may not be suited to the size of the object, i.e., its covering capacity is not large enough to cover the whole object with equal illumination. The remedy is to use an objective of higher numerical aperture or one of longer focus. With the latter alternative there will be a sacrifice of details.

Lack of sharpness in the negative is another trouble, giving flat or hazy prints. This may be due to the fogging of the plate during the exposure or development. Extra care should be taken to see that the plate is not unduly exposed to light during either exposure or development. The camera should be examined to see if it does not admit some light, or if there are not reflections from some of its internal surfaces. If no eye-piece is used, the trouble may come from the inner surfaces of the draw-tube. Lack of sharpness may come from over-exposure and under-development, or to the shifting of focus caused by vibrations in the apparatus or, if a vertical camera is used, to the settling of the microscope tube due to a faulty coarse or fine adjustment. The developer may not have been made up correctly, or it may have been used at a wrong temperature, or the development may have been prolonged too much. The fixing bath may be too old, or the plate may have been removed from it too soon. The trouble may arise from reflections from the back of an unbacked plate. Try a backed plate. It may also be due to too great changes of temperature when the plate is passed from one solution to another. See that the solutions and the water in which the plate is washed are at the right temperature. The fault may be due to mistiness on the lenses, especially on those of the eye-piece. Keep all lenses perfectly clean. The trouble may be due to the fact that the cover-glass is not of the right thickness for the corrections of the objective. With high powers, it may be due to the fact that the deficiencies of an achromatic objective have not been compensated for by using a proper ray filter, or the ray filter may not have been suited to the character of the object, or the proper plate for the colors of the object may not have been used.

Another common trouble in high-power work is due to diffraction fringes around the edges of the object (see page 222), due to the closing of the iris diaphragm of the sub-stage condenser too far, or to the fact that the object is too thick for the power of the objective used. There is no remedy for the latter except to use a lower-power objective and greater camera extension, but this results in loss of resolution in the details of the object.

Dirt on some of the components of the optical system may give rise to blotches or concentric circles in the print. See page 223.

If the negative is too thin all over, even though the details seem clear in it, but the print darkens too much in the background, the exposure was not long enough, the development was not sufficiently prolonged, the object was flooded with too much light or with some plates, the drying took too long. If the exposure or development was much too short, the background will be very transparent, the image will be weak all over, and there will probably be found on close inspection some areas that show no detail, but only clear glass. Such a negative is worthless.

If the background of the negative is too dense and the details do not come out in the print, the exposure was too long, the development was continued too long or, with some plates, the drying took too long. An over-exposed plate that has not been developed long enough is often flat and characterless.

If a negative that shows all the details is too thin to give a clear background in the print, it may sometimes be made into a good negative by intensification. Dissolve mercuric chloride in boiling water and when thoroughly cold add 1 c.c. of strong hydrochloric acid to each 200 c.c. of the clear solution. Wash the negative until all the hypo has been removed; if the plate is dry soak it in water. Then place it in the above solution until it turns uniformly white when examined from the front or through the back. Wash it half an hour. Then soak in water to each 200 c.c. of which 4 c.c. of strong ammonia have been added until the black color has been restored. Wash again thoroughly in water. Instead of ammonia, the results of which are strong but are liable not to be permanent, a 10% solution of sodium sulphite may be used, or the plate may be treated with pyro-soda developer or one of the hydroquinone developers.

When the negative is too dense to print in a reasonable time and does not allow the details to come out in the print, or does not give sufficient contrast or, on the other hand, when there is too great contrast and the denser portions need lightening, resort may be had to reduction. To increase the contrast, treat the negative after thorough soaking in water with Farmer's reducing reagent, which is simply very dilute fresh hypo solution (1 part saturated hypo solution and 8 or 10 parts of water) to which enough of a solution of potassium ferricyanide (red prussiate of potash) has been added to give a *very slight* color. It acts with great rapidity and must therefore not be used too strong. Under its action the plate becomes more transparent. The action must be watched, and, when it is judged to be sufficient, the negative is immersed in a large volume of water to stop it. This solution does not act much on the high lights but rather on the intermediate lights and increases the contrast, clears up fogginess and brightens up the negative. It must be used with judgment and care, or details will be lost in the more delicate parts. This is the most generally useful reducing reagent.

To reduce the densest part of a negative, soak thoroughly in water and treat with a solution made up of 5 to 10 grams of ammonium persulphate, 200 c.c. of water and 4 drops of strong sulphuric acid. When the action is judged to have proceeded nearly long enough, immerse the negative in a 5% solution of sodium sulphite to stop the action. Then wash thoroughly. This reagent reduces the contrast and is useful only when portions of the negative are too dense to show details. It may, of course, have happened with such negatives that the exposure, while correct for the other parts, was not correct for the dense parts of the object. In that case no treatment can bring out details that do not exist at all in the negative.

It should, of course, be the aim of the photomicrographer so to master exposure and development that intensification or reduction should be a last resort. It is usually preferable to make a new negative. There may, however, be occasions when the use of such processes is justifiable.

A photomicrographic negative should *never* be retouched. It is, however, permissible to stop up small holes that may have been caused by dust accidentally falling on the plate before development or to block out portions that it is not desired to show in the print. Other than this there should be no tampering with the negative.

The true *raison d'être* of a photomicrograph is to show fully all the structure of the object that can be seen under the microscope. Many scientific workers are satisfied if this is accomplished and pay no attention to the background. Consequently we see in many scientific books of high authority reproductions of photomicrographs with dark, muddy and unsightly backgrounds. By the use of the correct ray filter and by securing greater density in the negative by modifications of the developer or by prolonging the time of development, a photomicrograph that shows the details of structure perfectly and that has a clear white background can in the great majority of cases be produced, but sometimes perhaps at an unwarranted expense of time and material in trying several different ray filters and varying the degree of density in the negative. Very dense, slow printing negatives are required by some classes of objects to accomplish this result. The attainment of a clear white background appears to be impossible with some objects without sacrificing the details. This should never be done. It is far better to sacrifice artistic effects.

CHAPTER XIV.

PROJECTION AND DRAWING.

Projection of Microscopic Objects. — The apparatus arranged as shown in Fig. 67 makes an excellent projecting microscope. Special projection objectives and eye-pieces may be purchased, but many of those furnished with the ordinary equipment give good results. The distance to which the picture can be projected, and consequently its size and brilliancy, depends on the power of the objectives and eye-pieces used and on the intensity of the light. The illustration shows an Edison nitrogen filled Mazda C lamp of 250 watts capacity. This does very well, though the distance from the apparatus to the screen cannot be very great. The lamp is absolutely steady and requires no re-adjustment so that the undivided attention of the operator can be given to describing the slides. A reflector of correct

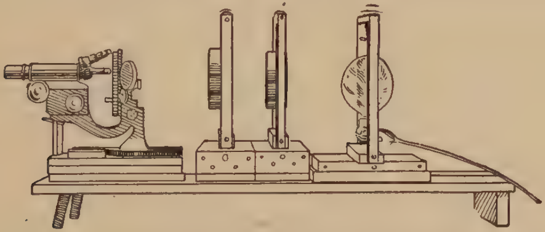

FIG. 67. — APPARATUS FOR PROJECTION WITH THE MICROSCOPE.

The adjustment of the condensing lenses is the same as that described for Fig. 58. The hood is removed to show the construction.

curvature to reflect the rays of light back onto the lamp filament adds much to the intensity of the illumination.

A support for the lamp may be constructed on the principle illustrated in Figs. 54 and 55. It is merely necessary to substitute for the piece *A*, Fig. 54, a square frame to carry the lamp. The lamp is then easily adjusted in the three directions as described for the auxiliary condenser. A well-ventilated hood should be fitted over the lamp, or better over the lamp and condensing lenses. With the light mentioned, clear pictures can be thrown to a distance of seven or eight feet with a 16 mm. objective and 6x eye-piece; with lower-power objectives, much farther.

A stereopticon arc lamp or a four or five ampere arc lamp may be used for projection work even on the alternating current, but they require constant attention and re-adjustment. With these lamps, good pictures are projected to a distance of twelve to fourteen feet with the 16 mm. objective and with the oil-immersion objective to a distance of four or five feet.

A skim-coated plastered wall makes one of the best of screens. A sheet of white paper is excellent. So also is a sheet of beaver board painted with two or three coats of a slightly blue tinted white enamel paint.

The apparatus is set up precisely as for photomicrography. The best results are obtained when two plano-convex lenses are employed, which may be telescoped together and held by a single lens support, but a single such lens may be used.

The powers of the objectives adapted to projection work are the 16 mm. and lower; they may be used with or without an eye-piece. The 16 mm. objective may be used with or without the Abbe condenser, but the Abbe can be used with lower powers only in case the front lens can be removed. If the Abbe is used, the auxiliary condensing lenses should be adjusted to give a clear image of the light source on the diaphragm of the Abbe. If no Abbe is used, the light must be focussed on the object on the stage.

Projection of Lantern Slides. — Fig. 68 shows an apparatus for the projection of lantern slides, when a more perfect equipment is not available. The support for the projecting lens is made on the principle already described for the supports for auxiliary condensers and the Mazda lamp and is adjustable in the three directions. A highly corrected projecting lens is necessary for the best results, but a good bi-convex lens such as is used in hand reading-glasses, with a diaphragm leaving only the central part for use, serves quite well. Some photographic lenses work very well. No diaphragm should be used on the condensing lenses.

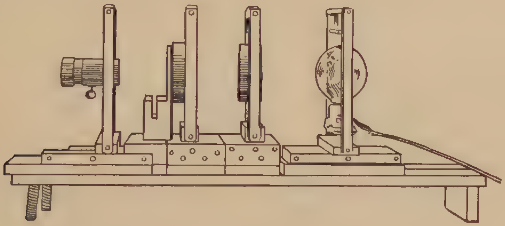

FIG. 68. — APPARATUS FOR THE PROJECTION OF LANTERN SLIDES.

With the hood removed. The support for ray filters shown in Fig. 66 is here used to hold the lantern slide.

This apparatus may be used when it is desired to project both microscope slides and lantern slides in the same lecture, as the change from microscope to projecting lens can be effected with very little loss of time.

Drawing with the Microscope Horizontal. — The apparatus as arranged for projection with the microscope may also be used in drawing objects as seen under the microscope.

Fig. 69 shows the arrangement. A wooden box has mounted on its top a piece of mirror at an angle of 45° with the optical axis. For this arrangement the mirror need not be more than one or two inches square, but for the arrangement shown in Figs. 70 and 72, it should be about six inches square. The thinner the glass of the mirror, the better, as troubles from double reflections are reduced. The light coming from the microscope is reflected at right angles by this mirror and passes down through a hole in the top of the box to the drawing paper placed on the bottom inside.

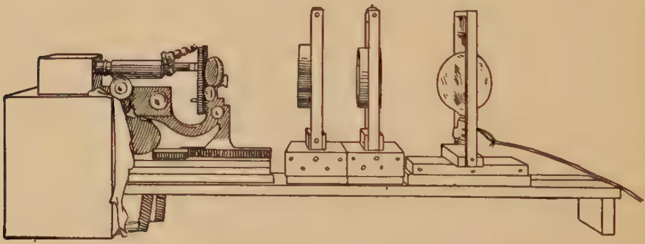

Fig. 69. — Apparatus for Drawing with the Microscope Horizontal.

With the hood removed.

The distance from the mirror to the drawing surface may be fixed at ten inches, or the drawing surface may be made adjustable. The side of the box to the left of the illustration is open and through this the image is easily drawn. The box should be wide enough to give free use of the arms and hands. The side of the box to the right is partially cut away to allow the mirror to be brought close up over the eye-piece. A curtain of black cloth is used between the box and microscope. If the work is done in a lighted room, a cloth should be used over the head like a focussing cloth. In a darkened room, or even in a dimly lighted part of the room, this is not necessary.

Drawing with the Microscope Vertical. — Fig. 70 shows an arrangement for drawing objects as seen under the microscope when in the vertical position. Far the most practical use of the microscope in drawing is to obtain representations of what is seen in fresh mounts that cannot be used on the microscope in the horizontal position.

For this arrangement the microscope is mounted on the special optical bed used in taking photomicrographs with the vertical camera. See Figs. 61 and 62. The same box that was used for Fig. 69 has a second piece of mirror one or two inches square inclined at an angle of 45° with the optical axis and connected with the other mirror by the bar B, the two mirrors being turned in opposite directions. Fig. 71 shows in diagram the path of the light rays. The light for drawing may be either the concentrated filament Mazda lamp, the tungsarc, or the electric arc. The two former are to be preferred for their steadiness; the latter for its intensity. The light must be focussed on the diaphragm of the Abbe condenser.

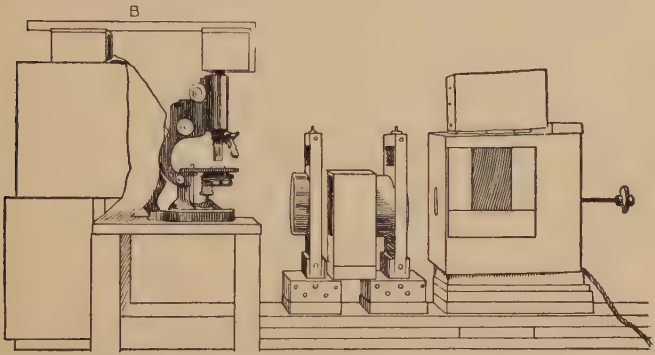

FIG. 70. — APPARATUS FOR DRAWING WITH THE MICROSCOPE VERTICAL.

A particularly good arrangement is shown in Fig. 72.

The Bausch and Lomb illuminator, placed in the upright position as for the vertical camera shown in Fig. 63, is used. This does away with one mirror. Convergent light gives the strongest illumination. The adjustments are precisely the same as for the vertical camera, and the apparatus shown in Fig. 64 may be used for this purpose.

The quality of the image depends on the quality of the mirror. With any mirror there is some distortion and haziness of the image. Naturally with mirrors such as described perfect results cannot be expected, but with large classes of objects quite satisfactory images are formed. For critical work with high powers the mirrors ought to be of the finest quality of polished metal or, still better, silvered prisms. Such mirrors and prisms and the apparatus designed for their use are expensive. The apparatus here described, while far from perfect, is much better than no apparatus for quick tracing of the significant structures

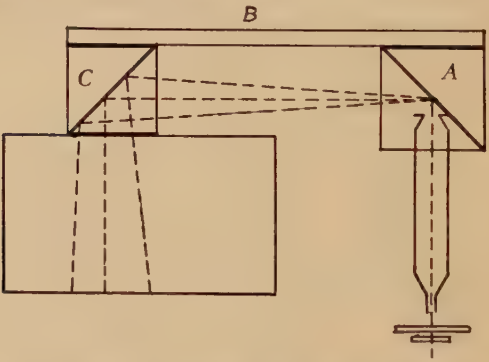

FIG. 71. — DIAGRAM SHOWING THE ARRANGEMENT OF THE MIRRORS.

And path of the rays in the apparatus arranged for drawing under the microscope in the vertical position. Mirror *A* is one inch square; mirror *C* is six inches square.

found in studying microscopic tissues. Try it and see if you do not agree. Its cost is practically nothing.

Drawings on Tracing Paper. — For making drawings on tracing paper, the following simple arrangement may be used. Nail together three pieces of board, ten inches longer than the height from the shelf, S, Fig. 72, to the eye-piece, to form a box without top or bottom and without one side. Stand this vertically over the microscope arranged as in Fig. 64 or Fig. 72 without drawing apparatus. Place a square of clear glass over the top, adjusting the height of the box so that the upper surface of the glass shall be exactly ten inches from the eye-piece. Slight differences of adjustment may be made by placing portions torn from a block of paper under the corners of the glass. Or, better, the glass may be let into a wooden frame so that its upper surface is flush with the wood, and the drawing surface thus provided may be made adjustable by elevating screws working through cleats secured to the sides of the box. This gives the additional advantage that the tracing paper may be held stretched in position by thumb tacks near the edges. Cover the open side of the box and the lamp support with a cloth curtain. The distance of the drawing surface from the eye-piece is not important in any of the arrangements for drawing unless one wishes to work at the standard distance of ten inches which has been adopted by common

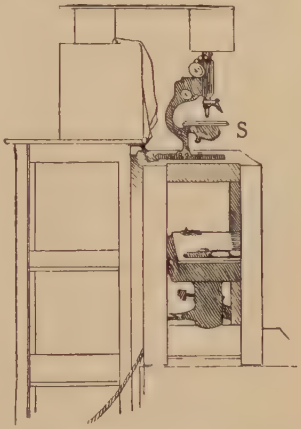

FIG. 72. — APPARATUS FOR DRAWING WITH THE MICROSCOPE VERTICAL.

Using the Bausch and Lomb illuminator.

consent for easy comparison. The drawing may be transferred in the usual way with carbon paper, or the traced drawing may be used as a negative and reproduced on a developing-out paper.

The magnifications of the drawings are easily determined by the use of a stage micrometer precisely in the same way as the magnifications of photomicrographs. See page 219.

With the drawing apparatus removed, the arrangement of lamp and microscope shown in Fig. 72 is excellent when doing visual work with the sub-stage dark ground illuminator (see page 303) or when using high-power eye-pieces and objectives which require intense illumination. For either drawing or visual work a 250- to 500-watt concentrated filament nitrogen filled Mazda lamp may also be used. A piece of ground glass or of the recently invented "daylite" glass, which gives effects nearly the same as daylight, may be interposed between the lamp and the sub-stage condenser to moderate the intensity. This glass is best placed close to the light source. The ground glass should not be used with the dark ground illuminator, as for this the strongest light obtainable is required.

Remove the drawing apparatus as shown in Fig. 70 or Fig. 72, support a single mirror at an angle of 45° immediately above the eye-piece, and an excellent arrangement is provided for the projection at short distances of microscopic preparations that can be used only when the microscope is in the upright position.

These simple arrangements for projection and drawing should appeal especially to teachers who use the microscope for class work. The distances of projection are sufficient for this purpose with small classes. The particular features of the objects shown can be pointed out so that it may be certain that the student *sees* what is being described, a fact that one cannot be at all sure about when the student is asked to look into the microscope. The attention of the whole class is centred at the same time on the same subjects of discussion, and there is much economy of time.

The drawing of objects is much less troublesome, less eye-fatiguing and even more accurate than with a camera lucida. When the outlines of the object have been traced, the drawing paper may be withdrawn, a fresh piece being substituted for it, and the details may be put in free-hand with the image comfortably before the worker. There are no difficult adjustments of apparatus and light, the fine adjustment screw is easily within reach when it is desired to bring different planes of the object into focus, the eyes and the hands are freer than when using the camera lucida.

CHAPTER XV.

MICROMETRY.

MICROMETRY is the art of measuring minute distances. The micrometer scale may be a scale ruled on glass like the stage micrometer in divisions of .1 and .01 mm. or .01 and .001 inch, which by means of the eye-piece micrometer disk may be made to furnish much smaller units of measurement by employing the different combinations of eye-pieces, objectives and tube lengths (see page 33), or it may be furnished by the pitch of a screw and a graduated screw head, as in the case of the fine adjustment, or by the graduated drum of the micrometer eye-piece or filar micrometer (see page 268), by the graduations on a mechanical stage, or by the graduations on a revolving stage for measuring angles.

The methods described in this chapter may be found repeatedly in the writings of various microscopists. It would perhaps be impossible to trace them to their originators and certainly it would be unprofitable to attempt to do it. They are given here with this general acknowledgment. It has not been at all the writer's aim to give an exhaustive treatment of the subject, but merely to group together the simplest methods of making the measurements that the practical user of the microscope may from time to time wish to employ.

Measurement of the Magnification of the Compound Microscope. — The simplest method of measuring the magnification obtained by the compound microscope with any combination of objective, eye-piece and tube length is as follows: Arrange the microscope as for projection (see Fig. 67). Use a stage micrometer, preferably one graduated in tenths and hundredths of the millimetre, projecting the image of the scale onto a paper screen set up exactly 250 millimetres from the eye-lens of the eye-piece. With dividers include any number of divisions of the projected image and, applying the dividers to an accurate millimetre scale, take the length. Divide this length by the number of divisions chosen, and we have the length of one division of the image. Divide this by the known value of one division of the stage micrometer used as the object, and the quotient is the magnification with the given combination of objective, eye-piece and tube length. What amounts to the same thing and is simpler, divide the length of any number of divisions of the image by the length of the same number of divisions of the same denomination on the stage micrometer.

The distance of normal vision for the unaided eye, 250 millimetres, is chosen by general agreement as the standard distance to which measurements are to be referred. The magnification may be measured at any other distance and with the tube at any other length in the same manner, but a statement of the conditions employed should then always be given with the measurements. By this method the magnification of a photomicrograph or drawing made at any distance is measured.

A table of the magnifications with all the possible combinations of objectives and eye-pieces and with the tube at standard length should be made at the normal projection distance and kept for reference. Other projection distances and other lengths of tube should be used only for special purposes, and the magnifications should then be determined for the particular case.

In a similar way the magnification may be obtained by measuring the image of the stage micrometer scale projected by a camera lucida, the drawing surface being 250 millimetres distant from the eye-lens of the eye-piece, or by using the drawing apparatus shown in Fig. 69, measuring the distance in like manner.

Another method of measuring the magnification of the compound microscope is by means of Sir A. E. Wright's *eikonometer.* The word eikonometer, derived from the Greek, means *image measure.* A description of this little instrument is given on page 275, and that description should be read in connection with the following paragraph. The writer has never used the eikonometer, but it is spoken highly of by competent microscopists who have. The description of the eikonometer and the directions for its use are taken from Wright's *Principles of Microscopy.*

Focus the microscope on a stage micrometer ruled in tenths and hundredths of the millimetre. Focus the Ramsden eye-piece of the eikonometer on the tenths millimetre scale in the eikonometer and place the instrument over the eye-piece on the microscope. Adjust the eikonometer in its tube so that the whole field of view of the microscope can be seen. The field-lens of the eikonometer projects the image of the stage micrometer scale into the same plane with the tenths millimetre scale in the eikonometer. Note how many divisions of the eikonometer scale correspond to some number of divisions of the stage micrometer scale. Divide the former by the latter, and we have the magnification produced by the combined effect of the objective, eye-piece and tube length employed. It must be remembered though that the divisions of the eikonometer scale are to be counted as millimetres, since each division counts as a millimetre seen at the distance of normal vision, i.e., 250 millimetres.

Suppose 6 divisions of the eikonometer scale correspond to 4 of the hundredth millimetre divisions on the stage micrometer. Then, 6 mm. ÷ .04 mm. = 150 = the magnification. Or, suppose 5 of the divisions of the eikonometer scale correspond to 1 one-hundredth division of the stage micrometer. Then, 5 mm. ÷ .01 mm. = 500 = the magnification.

Measurement of Objects under the Compound Microscope. — The most accurate means of measuring the size of objects under the microscope is a micrometer eye-piece or filar micrometer. The next most desirable means is an eye-piece micrometer, — a disk of glass with equally spaced rulings placed ruled side down on the diaphragm of the eye-piece. The method of using this as well as the method of determining the values of the graduations on the eye-piece micrometer in terms of the graduations on the stage micrometer for different combinations of eye-piece, objective and tube length are given on page 34.

The micrometer eye-piece differs from the eye-piece micrometer disk only in that its scale is movable. The scale in the Spencer micrometer eye-piece is 5 mm. long, each millimetre being divided into quarter millimetres. Each complete revolution of the screw that actuates the scale moves it over one of these divisions or .25 mm. The drum head of the screw is divided into 100 parts. Turning the drum through one of its divisions therefore moves the scale .0025 mm. The valuations of the divisions of the scale of the micrometer eye-piece in terms of the divisions on the stage micrometer are determined as are those of the eye-piece micrometer disk (see page 34), i.e., the value of one space on the stage micrometer is divided by the number of spaces and the fraction of a space on the micrometer in the eye-piece that exactly cover it. These valuations should be worked out with the tube at standard length for each objective that is to be used and a record kept for reference. If the tube is to be used at any other than the standard length, a special determination must be made for that length. Except for this use, the stage micrometer is no longer needed; also no further attention need be paid to the *actual* values of the divisions on the scale in the eye-piece. An example will make the method of using this instrument clearer. Suppose a particular diatom is to be measured. Focus the eye-piece, which is of the Huyghenian type, on the scale and by revolving it make the scale coincide in direction with the dimension to be measured. By adjusting the slide on the stage or moving the scale, make the zero line just touch the edge of the object. Suppose now it is found that the object covers five of the divisions of the scale and a portion of another division. Set the index at zero. This can be done without moving the scale. Revolve the drum until the fifth line of the scale just touches the other edge of the object. Suppose we have revolved the drum through 35 of its divisions. We now know that the object covers 5.35 of the divisions of the scale. Consulting our table of valuations, we find the value of one division of the scale with the objective used is .001 mm. The object therefore measures .001 mm. × 5.35 = .00535 mm. = 5.35 μ, a result that is much more accurate than it

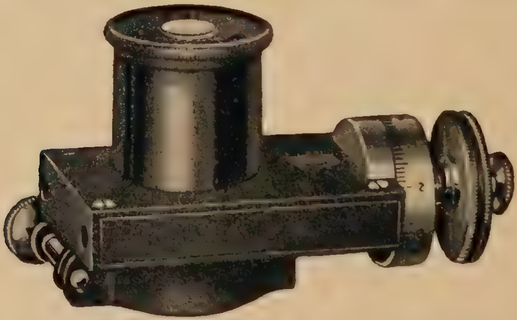

FIG. 73. — SPENCER MICROMETER EYE-PIECE.

would have been, if it had been necessary to *estimate* rather than actually *measure* the partial division, as must be done when an eye-piece micrometer disk is used.

The Bausch and Lomb filar micrometer has a fixed scale ruled in spaces of .5 mm. A fine wire parallel to the divisions of the scale is moved by a screw whose drumhead is divided into 50 equal parts. One complete revolution of the drum moves the wire over one space on the fixed scale, i.e., over .5 mm. The turning of the drum through one division advances the movable wire .01 mm. In some types a fine fixed wire, in other types two fixed wires at right-angles to each other, run through the centre of the field to assist in locating the object to be measured. Some types show in the field of view also a fixed comb with pointed teeth, each interval between the teeth indicating the distance the movable wire is advanced by one complete revolution of the drum. Some types have the drum divided into 100 parts. The Ramsden eye-piece can be focussed on the scale and wires.

To use the filar micrometer revolve it to make the direction of the scale coincide with the dimension to be measured. Adjust the movable wire so that it just touches the edge of the object. Then by turning the drum move the wire until it just reaches the other edge of the object, noting on the fixed scale and the revolutions of the drum the number of spaces and final fraction of a space covered by the object. Suppose it required four complete revolutions and one partial revolution through 20 of the divisions on the drum. Then the object covers 4.4 of the divisions of the scale. The value of one division of the scale, determined precisely as for the micrometer eye-piece, multiplied by 4.4 gives the length of this particular object.

The micrometer eye-piece and filar micrometer, being capable of such accurate adjustment to the object, are especially desirable in measuring objects so minute that they do not fill even one division of the eye-piece scale. The division of the drum into 50 or 100 parts provides the means of dividing the divisions of the eye-piece scale in the same way and secures delicacy of measurement to the same extent. It is also possible to estimate a fractional part of one of the divisions on the drum to secure even greater accuracy.

Another very convenient and quite accurate method, and one much to be recommended, may be applied with only a stage micrometer. Arrange the microscope as for drawing (see Fig. 69). If the magnification of the microscope for the particular objective, eye-piece, tube length and projection distance used has not already been determined, that determination must now be made (see page 264). Then proceed as follows: —

Measure the magnified image of the object as projected onto the drawing surface and divide this by the magnification for the particular combination of eye-piece, objective, tube length and projection distance employed, and we have the measurement of the object.

Or, with the microscope vertical, use the arrangement for taking photomicrographs with the microscope in that position, and measure the magnified image on the ground glass (see Figs. 60, 61, 62, 63). Or, again, instead of the camera, use the arrangement described on page 261 for making drawings on tracing paper. In either case, determine as before the magnifications for each combination of eye-piece, objective, tube length and projection distance. Proceed as in the last paragraph.

The distance of the drawing surface or of the ground glass from the eye-piece is of no importance in making the determinations of the magnifications, provided the same distance is always used in measuring the images.

Coarse objects may also be measured roughly with a mechanical stage. With mucilage affix a very fine hair to the upper surface of the diaphragm of an eye-piece so that its end shall be at about the centre of the field of view. Using this eye-piece, see that the end of the hair is in exact focus. If it is not, unscrew the eye-lens until it is, or properly adjust the hair in the plane of the diaphragm. Bring one edge of the object to be measured directly under the end of the hair, revolving the eye-piece, if necessary. Take the reading of the mechanical stage. Then bring the opposite edge of the object under the end of the hair and again take the reading of the mechanical stage. The difference of the two readings is, of course, the measurement desired.

A cover-glass with a diamond scratch across the centre, or a very minute ink-spot near the centre, placed scratched or spot side down on the diaphragm of the eye-piece, may be used instead of the hair. This method is only a slight modification of the old-time method with crossed hairs in the eye-piece.

As the vernier on the mechanical stage usually reads to only .1 mm., the limit of accuracy of this method is, of course, .1 mm. or 100 microns. It is evident that the method cannot be used for very delicate objects or when a high degree of accuracy is desired. Another objection is that, unless the stage of the microscope revolves, it is possible to measure only circular objects or those favorably located on the slide so that the dimensions to be measured coincide with the directions of movement of the mechanical stage. It is, however, a convenient means for rough measurements and is much employed.

The eye-piece with inserted hair or a cover-glass with ink-spot also provides a very convenient *pointer* when the worker wishes to call the attention of another person to some particular object in the field of view. Such a pointer has been used from early times in microscopical study.

To measure an object with Wright's eikonometer, first determine the magnification of the microscope with the objective, eye-piece and tube length employed, as already described. Then place the object to be measured on the stage and adjust the eikonometer over the eye-piece. Read the dimensions of the image of the object as measured by the eikonometer scale, remembering that these divisions count as millimetres. Divide these measurements by the magnification of the microscope as used, and we have the dimensions of the object. For instance, if an object measures 4 divisions of the eikonometer scale and the magnification of the microscope as used is 500, then 4 mm. ÷ 500 = .008 mm. = 8 μ.

Measurement of the Field of View of the Compound Microscope. — This may be done directly with a suitable stage micrometer. With the proper adjustments made, the field well lighted, and the rulings of the micrometer scale sharply focussed, read the diameter of the field on the scale just as one would measure a circle with a foot rule. Take the measurement with tube at standard length. It is also instructive to pull the draw-tube out to various lengths and note the changes in the diameter of the field. If the scale on the stage micrometer is not long enough to measure the diameter of the field of low-power objectives, employ an eye-piece micrometer disk graduated to tenths of the millimetre placed on the stage as an object.

Measurement of the Working Distance of an Objective. — The most accurate method with high-power objectives is with a fine adjustment screw that has a graduated head. Focus the objective on a particle of dust, ink-spot or scratch on a slide. Then carefully lower the objective until it touches the slide. Take the reading of the fine adjustment screw. Now focus up with the fine adjustment until the dust, ink-spot or scratch is again in accurate focus, noting the number of full revolutions and the final partial revolution of the screw, and from the known valuations of the fine adjustment calculate the amount the objective has been raised.

With low-power objectives, whose working distance is greater than the limits of the fine adjustment screw, Gage suggests to run a wedge-shaped piece of wood between the objective and slide. Mark with a pencil as nearly as possible the point where the front lens touches the wood, and measure the thickness of the wedge at that point. This will give the *approximate* working distance.

When a cover-glass is used, the working distance is diminished not by the full thickness of the cover, but by two thirds of that thickness (see page 278).

Measurement of the Initial Magnification of an Objective. — Place an eye-piece micrometer disk with scale divided into tenths of a millimetre in position in an eye-piece and remove the field-lens of the eye-piece. Place a stage micrometer with scale ruled in tenths and hundredths of a millimetre on the stage. Use the tube length for which the objective was corrected. Observe how many divisions of the eye-piece micrometer cover one division of the stage micrometer. Take the measurements as near the centre of the field as possible to avoid the distortions of the lines of the scale which are very evident when the field-lens of the eye-piece is removed. The quotient of the former by the latter gives the initial magnification of the objective according to the ratings of American manufacturers. For instance, suppose that .1 mm. on the stage micrometer is covered by 1 mm. on the eye-piece micrometer. Then 1 mm. ÷ .1 mm. = 10 = initial magnification of the objective. Or, suppose .1 mm. of the stage micrometer is covered by 6 mm. on the eye-piece micrometer. Then 6 mm. ÷ .1 mm. = 60 = the initial magnification of the objective used.

Of course, a stage micrometer and an eye-piece micrometer with scales divided in decimals of an inch may be used in the same way. The only point of importance is that the two scales must be in the same denominations.

Eye-pieces vary somewhat in accuracy. It is advisable to make the measurements with several when possible.

Measurement of the Power of an Eye-Piece. — Measure the magnification of the combined eye-piece and objective, i.e., of the microscope, as previously described. Measure the initial magnification of the objective by the method given in the last section. The quotient of the former by the latter gives the power of the eye-piece.

This method gives an easy means of determining the power of an eye-piece when the initial magnification of the objective is known, or the initial magnification of an objective when the power of the eye-piece is known.

Measurement of the Magnification of a Simple Microscope. — If one has two good eyes, both of which focus at the normal distance of 250 mm., he may determine the magnification of a simple microscope as follows: Place a ruler on the table. At a height of 250 millimetres, measured from the top surface of the lens to the scale on the ruler, support the lens to be examined. Hold another ruler, graduated like the first, beneath the lens, raising it until the graduations are focussed by the lens. With one eye focus sharply the markings on the second ruler, bringing the eye near enough to the lens to obtain the largest field of view possible, at the same time observing with the other eye the markings on the first ruler. Determine what number of divisions on the first ruler correspond to one division of the same denomination on the second ruler. Divide the latter by the former, and we have the magnification of the lens. The lighting must be such that the two rulers are equally illuminated. It also required some mental effort to see the two scales at the same time. It is best to keep closed the eye by which the scale of the first ruler is to be observed until the other eye, held as close as possible to the lens, has focussed the scale on the second ruler, and then suddenly open the closed eye. This method is given by several reliable authorities. The writer cannot apply it.

Another method is that with Sir A. E. Wright's eikonometer, a sectional view of which is shown in Fig. 74.

E is a plano-convex lens which focusses on a scale ruled to tenths of a millimetre placed at *F*. *ABCD* is a tube into which the instrument can be adjusted so that the lens *E* serves as a field-lens to receive the image formed at the eye-point of the lens under examination and in such a manner as to take in the whole field of view. This magnified aerial image is projected by the lens *E* onto the scale at *F*, reduced to one tenth of its size as seen by the unaided eye placed at the eye-point of the lens. *G* is a Ramsden eye-piece which focusses on the scale at *F*, magnifying the tenth millimetre divisions so that each division corresponds to one millimetre placed at 250 millimetres from the eye, i.e., it restores the image to the same size as when seen directly with the lens under examination. The scale at *F* and the image of the object under the lens are now seen in the same plane, and it amounts to the same thing as applying a millimetre scale directly to the image.

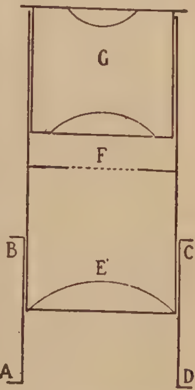

FIG. 74.—WRIGHT'S EIKONOMETER

To measure the *magnifying power* of a lens with the eikonometer, support the lens above a ruler graduated in millimetres at such a height that the millimetre scale is in focus and gives the largest image possible. Adjust the eikonometer over the lens so as to take in the whole field of view, and note how many divisions of the eikonometer scale cover one division of the image of the scale used as object. The number thus obtained is the magnifying power of the lens under examination.

To measure an *object* with a simple microscope and an eikonometer, make the adjustments as above, substituting the object for the millimetre scale, and read off the dimensions of the image on the scale at F, counting the tenth millimetre divisions as millimetres. Divide the dimensions of the image by the magnifying power of the lens, and we have the dimensions of the object.

To Measure the Thickness of the Cover-Glass on a Mounted Specimen. — It is sometimes desired to determine the thickness of a cover-glass on a mounted specimen. As the refractive indices of balsam and the cover-glass are practically the same, if the object is mounted in balsam and not in contact with the cover-glass, the datum desired is the distance from the object to the top surface of the cover. With this found we know whether the draw-tube should be lengthened for a cover-glass that is thinner, or shortened for one that is thicker than that for which the objective was corrected.

This determination can be made if the fine adjustment screw has a graduated head. Focus with a dry objective — say a 3 or 4 mm. — on the object and take the reading of the fine adjustment. Then focus up onto a speck of dust on the top surface of the cover-glass noting the number of turns and fraction of turns of the fine adjustment screw. From the known values of the fine adjustment calculate the amount the objective has been raised. This is the *apparent* thickness of the cover-glass.

Now it is a well-established principle of Optics that rays coming from one medium into another medium, in a direction perpendicular to the surface bounding the two media, form an image at a distance from the bounding surface which is the distance of the object from that surface multipled by the index of refraction of the medium into which the rays pass with reference to the medium from which the rays come.

Although this principle is demonstrated in books on Optics as mathematically true for all media and for rays passing in either direction between the media, it seems to be of sufficient interest to warrant the reproduction here of the proof in a simplified form and applied to the particular case under consideration.

Let AB, Fig. 75, be the top surface and CD the bottom surface of a cover-glass with air above and balsam below. Let E be a point on an object in contact with the lower surface of the cover-glass or in the balsam below, i.e., practically in the glass. Let EF be a ray incident on AB at F. Since the ray is passing from a denser to a rarer medium, it is refracted away from the perpendicular HK and takes some such direction as FG (see pages 6 and 7). As far as this ray helps to form it, the image appears to one looking at it from above at E' where GF produced downward crosses the perpendicular EM drawn from the point E to AB. EM and HK are parallel.

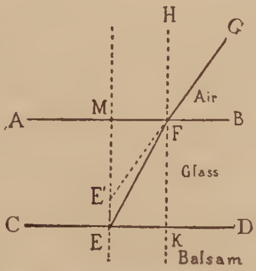

FIG. 75. — EFFECTS OF A COVER-GLASS.

The angle of incidence $EFK = \angle FEM$.

(Alternate-interior angles of // lines).

The angle of refraction $HFG = \angle FE'M$.

(Exterior-interior angles of // lines).

Let n represent the relative index of refraction of a ray passing from the cover-glass into the air. Then

$$n = \frac{MF}{EF} \div \frac{MF}{E'F} = \frac{E'F}{EF} \text{ (See page 7.)}$$

If now EF be made to approach the position of EM as a limit the ratio $\frac{E'F}{EF}$ holds constantly true for all positions, and, when EF takes the position of EM, $\frac{E'F}{EF}$ becomes $\frac{E'M}{EM}$. In this position of the ray we have the condition under consideration, i.e., as one sees the image through the microscope, he is looking down perpendicularly on the surface of the cover-glass that bounds the two media.

Substituting $\frac{E'M}{EM}$ for $\frac{E'F}{EF}$, we have

$$n = \frac{E'M}{EM}$$

$$E'M = nEM.$$

The absolute index of refraction of the air is 1 plus a fraction so small that it is usually neglected. The absolute index of refraction of the cover-glass is 1.52 or approximately 3/2. Therefore n, the relative index of refraction of rays passing from the cover-glass into air, is $1 \div \frac{3}{2} = \frac{2}{3}$ (see pages 6 and 7). Therefore

$$E'M = \tfrac{2}{3}\, EM.$$

That is to say, the *apparent* distance of the object from the upper surface of the cover-glass is $\frac{2}{3}$ of its *real* distance. We must, then, multiply the *apparent* thickness of the cover-glass as we have determined it with the fine adjustment by $1\frac{1}{2}$ to obtain its *real* thickness.

These facts may be verified by measuring by this method a cover-glass the thickness of which has been determined with a cover-glass gauge or a wire gauge such as is used by mechanics in measuring the diameters of wires. Focus on specks of dust or ink spots placed on the opposite sides of the cover-glass. Work with great care and repeat several times to verify the results.

This method gives only approximate results, but results that are sufficiently accurate to tell us in which direction to move the draw-tube to obtain the proper tube length to give the best image. The exact tube length must be determined by careful trial and to do this accurately requires considerable experience. The method is not especially to be recommended and is given here rather to call attention of inexperienced workers to the effects of the refraction of the cover-glass and to prevent the error sometimes made of mistaking the *apparent* thickness of the cover as measured by the fine adjustment for the *real* thickness. It is usually easier and more desirable to judge of the correct tube length by the appearance of the object as the length of the tube is changed, but the photomicrographer occasionally finds it desirable to measure the thickness of the cover-glass on some valuable mounted specimen that he wishes to photograph.

CHAPTER XVI.

THE SUB-STAGE CONDENSER.

There is little difference of opinion among experts that a highly corrected sub-stage achromatic condenser is of the greatest importance in *critical* work with high powers. These condensers have been brought in recent years to a very high degree of perfection. The chromatic aberration has been reduced to the minimum. The spherical aberration has been so highly corrected that it is claimed for some that the aplanatic cone — i.e., the cone of light for which all rays come to practically the same focus — is the full numerical aperture of the condenser. For others 90% to 95% of the numerical aperture is claimed. Unfortunately such sub-stage condensers are very expensive, and the ordinary worker has to be content with a less perfect means of illuminating the object.

For the Abbe chromatic condenser — the type ordinarily supplied with microscopes of moderate cost — an aplanatic cone of only about .5 is all that it is possible to secure. Because of its low cost and because the results obtained in ordinary non-critical work are quite satisfactory, this chromatic condenser has come into very extensive use. As it is uncorrected, neither the centring nor the focussing must be done with as much accuracy as with a highly corrected condenser. It is, therefore, much easier to use than the corrected types.

Whatever type of condenser is used, it is highly important to know how to secure the best results with it. A few points will be considered here.

Centring the Sub-Stage Condenser. — It is important to know that the sub-stage condenser is accurately centred. The expensive achromatic condensers are provided with centring screws to accomplish this. In the case of the less expensive microscopes, the Abbe condenser is not so provided, but is centred by the manufacturers. However, if the Abbe is turned in the sub-stage fitting, the centring *may* not be perfect. To test it proceed as follows: Focus on some object, see that the light is accurately centred, and remove the object from the field of view. Close down the iris diaphragm of the condenser until the back lens of the objective is only partially filled with light, as may be seen by removing the eye-piece and looking down the tube of the microscope at the image of the sub-stage iris diaphragm in the back lens of the objective. While looking at the back lens of the objective, gradually open the iris diaphragm of the condenser. The illuminated circle should increase evenly until it exactly fills the back lens of the objective, and the circle should be perfectly uniform. Instead of looking at the back lens of the objective, its image — called the Ramsden circle — may be observed by looking from a distance of about ten inches at the eye-point above the eye-piece with a magnifying glass. If the illuminated circle is not exactly in the centre of the back lens, the condenser is not accurately centred and should be adjusted with the centring screws, turned around in its fitting, or sent to the manufacturers.

If a revolving nose-piece is used, the sub-stage condenser, when centred for one objective, is not necessarily centred for other objectives. These nose-pieces are seldom of a high degree of perfection. In work demanding great accuracy, clutch nose-pieces (see Fig. 48) are much to be preferred, or a nose-piece may be dispensed with and the objective screwed directly to the lower end of the body tube.

Focussing the Sub-Stage Condenser. — The importance of accurately focussing the sub-stage condenser for photomicrography has been discussed on page 223. For visual work with the Abbe condenser, because of the large diameter of the front lens and since the rays of light, owing to the spherical aberrations of the uncorrected lens, do not come to a very definite focus, it is not quite so important, except when critical work of great exactness is attempted, and yet the best results cannot be secured in any work unless the Abbe is in reasonably accurate focus. The sub-stage condenser is said to be in focus when it forms a sharp image of the light source in the plane of the object on the stage. It is made to work with parallel light. Therefore when using daylight always use the plane mirror with high-power objectives. With low-power objectives, unless the unobstructed light from the sky can be used, images of the window bars, trees and other objects show in the field of view when both the objective and condenser are focussed on the object on the stage. To do away with these, use the concave mirror or lower the condenser until they disappear.

The following experiments for focussing the condenser for artificial light will amply repay for the trouble of making them. Place a kerosene lamp so that the flame shall be about eight inches from the mirror of the microscope. Use the plane side of the mirror. Focus on some small object with the 16 mm. objective. Turn the edge of the flame towards the mirror. Adjust the Abbe condenser until the image of the flame is also focussed on the object, the object and image of the flame being seen at the same time. Adjust the Abbe until the edge of the flame is as sharp as it can be made. Then by means of the mirror see that the image of the flame lies centrally across the field of view. Set the iris diaphragm of the Abbe so that the back lens of the objective, viewed by removing the eye-piece and looking down the tube of the microscope, is three fourths filled with light. These are the conditions laid down by Mr. E. M. Nelson, one of the most eminent of English microscopists and accepted by experts generally, for the best performance of both sub-stage condenser and objective. The light is said to be in *critical* adjustment, and a *critical image* of the object is formed. The field, however, is not fully illuminated with the low-power objectives, and only a very small part of it can be used. Some way must be found to light the whole field evenly. This is done by a *slight* departure from *critical* illumination and is usually effected by lowering the Abbe *just sufficiently* to spread the image of the flame over the whole field of view. Other ways are to use the concave instead of the plane mirror, turn the flame broad side on, or turn the mirror slightly to one side so as to throw the image of the flame out of the field.

When the sub-stage condenser is focussed for critical illumination, it is focussed for a high-power objective. With the adjustments made for critical illumination, change from a low-power to a high-power objective. The image of the flame now fully and evenly lights the field, and, if the image of the flame was accurately focussed with the low-power, no change in the position of the Abbe is necessary. Owing, however, to the size and thickness of the flame and the lack of corrections in the Abbe, it is difficult to be sure when its image is *exactly* in focus, so that, whatever objective is used, *slight* adjustments of the condenser may be necessary to obtain the greatest brilliancy of illumination. The iris diaphragm of the sub-stage condenser is then made use of to control the light according to the character of the object and the personal preferences of the observer, but for each change in the opening in the iris the focussing of the condenser should be tested to see if it is accurate. Only in this way can the most perfect resolution of a delicate object be secured. As the iris diaphragm is closed, the numerical aperture of the cone of light passing through it is reduced, and it will be noticed that better illumination and greater penetration are obtained by lowering the condenser. Conversely, if the iris is opened wider, the condenser must be raised for the best effects. On changing slides, adjustments of the sub-stage condenser are necessary, owing to the variation in the thickness of slides.

When one has made a study of the correct focussing of the Abbe, it becomes easy to judge of its proper position by merely observing the intensity of the light in the field of view. The Abbe is brought up into contact with the slide and then slightly lowered until the field is evenly lighted with the most intense illumination attainable, which again is moderated by the use of the iris diaphragm until the details of the object are brought out with the greatest sharpness.

A study of the effects on the illumination as the iris diaphragm of the condenser is opened or closed and the condenser raised or lowered, using the microscope arranged in the horizontal position as for photomicrography (see page 222), observing the light effects on a paper screen set up in the place of the camera, gives a very clear idea of the adjustments of the condenser and its iris diaphragm. This study should be made first without an auxiliary condenser; afterward with one, to see what the effects produced by the auxiliary condenser are.

The Passage of the Light from the Condenser to the Object on the Slide. — When light passing from a rarer to a denser medium strikes the bounding surface, it passes as a whole through that surface into the denser medium. But when it passes from a denser to a rarer medium, only so much as is included within the critical angle passes (see page 8). The critical angle is different for different pairs or media and depends on the relative index of refraction of the media (see page 7). The sine of the critical angle for any pair of media may be found by dividing the absolute index of refraction of the rarer medium by the absolute index of refraction of the denser, and the *angle* by looking up the sine thus obtained in a table of natural sines. All rays that strike the bounding surface at an angle greater than the critical angle are totally reflected in the medium whence the light comes. The rays which strike the surface at the critical angle pass along that surface. Rays striking the surface at angles less than the critical angle pass and spread out into an angle of 180° in the rarer medium.

In considering the sub-stage condenser, we are concerned with the critical angle for glass and air, glass and water, and glass and immersion oil.

The sine of the critical angle for glass and air is $1 \div 1.52 = .6578$ (see page 7). Consulting a table of natural sines, we find that approximately .6578 is the sine of 41°. In this case only light that strikes the lower side of the upper surface of the front lens of the condenser within the angle of about 41° on each side of the normal can pass and help form the image.

The sine of the critical angle for glass and water is $1.33 \div 1.52 = .875$. The table of natural sines gives approximately .875 as the sine of 61°. It is evident that much more light passes through the bounding surface.

The sine of the critical angle for glass and immersion oil is $1.52 \div 1.52 = 1$. Now 1 is the sine of 90°. That is, all the rays that can come from the condenser pass the bounding surface.

The perfection of the image depends on the number of rays that help form it. It is evident then that the best image will be formed when immersion oil is used between the sub-stage condenser and the slide; the next best when water is used; and with air there is a very considerable loss of light.

With dry objectives the greatest possible theoretical numerical aperture is 1. For, $N.A. = n \sin. u.$ (see page 28). The greatest possible value of u is 90°. Hence $N.A. =$ Index of Refraction of Air $\times \sin. 90° = 1 \times 1 = 1$. In making objectives the full angular aperture of 180° cannot be attained. It is also true that the full numerical aperture of a dry objective can in practice seldom be used, as too much light tends to obscure the image. The numerical aperture of the sub-stage condenser for ordinary uncritical use with low powers need not exceed about two thirds of the numerical aperture of the objective, as the light diffracted and reflected by the object helps to supply the full numerical aperture of the objective. The sub-stage condenser, if used dry, no matter what its construction is, can only supply a numerical aperture limited by the critical angle for glass and air. That is, the $N.A = n \sin. u = 1 \times \sin. 41° = 1 \times .6578 = .6578$. This is sufficient for a numerical aperture of 1, i.e., for dry objectives, but it is not sufficient for immersion objectives whose numerical aperture is greater than 1, and in the use of which there is not sufficient light to flood the image, and the diffraction and reflection from the object are not enough to satisfy in any marked degree the numerical aperture of the objective.

With water as the immersion fluid, the $N.A. = n \sin. u = 1.33 \times \sin. 61° = 1.33 \times .875 = 1.16$. With immersion oil, $N.A. = n \sin. u = 1.52 \times \sin. 90° = 1.52$.

If now a sub-stage condenser with a numerical aperture of 1.20 or 1.40 is used, its whole numerical aperture is available for illuminating the object only when immersion oil is used to connect it with the under surface of the slide.

In ordinary work with dry objectives, as in the study of animal and vegetable tissues, the use of two thirds to three fourths of the numerical aperture of the objective and in some cases even less is found to give the clearest images, —

images not so flooded with light that the details are obscured. This reduction of the numerical aperture is secured by shutting down the iris diaphragm of the sub-stage condenser, and the approximate fraction of the numerical aperture employed may be estimated by removing the eye-piece and looking down the tube of the microscope at the back lens of the objective or by looking at the Ramsden circle above the eye-piece with a magnifying glass. If the back lens is filled with light, the whole numerical aperture is being utilized. If the back lens has a dark area around it, the whole numerical aperture is not in use, and one may judge of the fractional part employed by estimating the fractional part of the back lens that is illuminated. In reducing the numerical aperture by shutting down the iris diaphragm, care must be taken not to carry it so far that diffraction fringes are formed about the object. This is especially important in photomicrography, as the diffraction fringes become much more evident in the photomicrograph than they are visually. Diffraction fringes of particles of dust on the object or on some of the components of the optical system ruin the photograph.

With the high powers used in critical work, as in the study of bacteria, the amoeboid movements of white blood corpuscles, the cilia of microörganisms, granules in cells, or the fine details of the markings on diatoms, and especially in the photography of such details, it is highly important that the full numerical aperture of the objective be available. In photographing some such objects, however, especially living microörganisms, *slight* diffraction fringes give more distinct images. The iris diaphragm is then purposely shut down to produce them, but great care must be taken not to overdo it.

To see the effect of connecting the sub-stage condenser with the under surface of the slide with immersion oil, focus with the immersion objective on some suitable object and focus the sub-stage condenser used dry to obtain the most brilliant illumination possible. Remove the eye-piece and observe that the back lens of the objective is only partly filled with light. Then make oil connection between the condenser and the slide. Observe that now the back lens of the objective is more fully filled with light; also that the fine details of the object are now more clearly seen.

The Working Numerical Aperture. — Another matter demands consideration. The *working* numerical aperture of the objective and sub-stage condenser taken together is one half the sum of the numerical aperture of the objective and of the aplanatic cone of the condenser. For instance, using a 4 mm. objective with a numerical aperture of .85 and an achromatic condenser with an aplanatic cone of .9, the *working* numerical aperture is $\frac{1}{2}(.85 + .9) = .875$, which satisfies the requirement. But with the same objective and an Abbe chromatic condenser, the *working* numerical aperture is $\frac{1}{2}(.85 + .5) = .675$, which is too low for critical work, but serves very well in non-critical work.

Again, if an oil-immersion objective having a numerical aperture of 1.25 is used with a very highly corrected condenser of a numerical aperture of 1.40, the *working* numerical aperture is $\frac{1}{2}(1.25 + 1.40) = 1.32$, or approximately so, if the aplanatic cones are nearly equal to the numerical apertures, as they are claimed to be with objectives and condensers of the highest quality. But with the same objective and an Abbe chromatic condenser, the *working* numerical aperture is only $\frac{1}{2}(1.25 + .5) = .875$. The superiority of the highly corrected sub-stage condenser for critical work is evident. In photomicrography, especially, work of the highest perfection demands its use as well as the use of highly corrected auxiliary condensers. In this book which has been written for those who possess only the ordinary equipment, photomicrographs made with the Abbe sub-stage condenser and with uncorrected auxiliary condensers are shown.

Contrasting Views. — The foregoing considerations have led to the now commonly accepted view that a highly corrected sub-stage condenser is necessary for the most perfect results in *critical* work. On the other hand, Sir A. E. Wright, an eminent authority on the optics and theory of the microscope, in his *Principles of Microscopy*, edition of 1907, page 185, contends that the Abbe chromatic condenser furnishes at nodal points formed by the intersection of rays from different points on the light source light that cannot be distinguished in color or in any other particular from that furnished by a highly corrected condenser from its focal points, and that it is at an angle as great or even greater than the light from a highly corrected condenser. In other words he contends that both the chromatic and the spherical aberrations are very completely corrected automatically at the nodal points. In his view the uncorrected Abbe works with quite as great efficiency and at quite as high a numerical aperture as does the highly corrected condenser of the same numerical aperture. He proves these contentions to his own satisfaction by diagrams and illustrations. The nodal points are brought into the plane of the object on the slide by slightly lowering the Abbe, and there are certain ranges within which this can be accomplished. Unless the highly corrected condenser is centred and focussed with the greatest nicety, the advantages to be secured by it are lost, and such a condenser is then inferior to the uncorrected Abbe. Wright's conclusion is that the Abbe is quite as efficient as the highly corrected type, and that, under the conditions of ordinary microscopic work, the results are not appreciably different from those obtained with the highly corrected type.

Hind and Randles, *Handbook of Photomicrography*, page 111, say " If delicate work, done with an Abbe Condenser, is compared with similar work with the more expensive types, it is extremely difficult to find any difference."

Barnard, *Practical Photomicrography*, page 47, says " It [the Abbe condenser] may for medium powers be used satisfactorily for photomicrography. With direct light it has comparatively few limitations; but when used as an oblique illuminator — that is, with its central rays stopped out — it breaks down and fails to fulfil its purpose in the way that better corrected condensers will do."

In contrast with this view, Spitta, *Photomicrography*, page 92, says, " The ordinary so-called Abbe condenser is a chromatic one, and is of no use in photomicrography." And Dr. Dallinger, the reviser of Carpenter's *The Microscope and its Revelations*, says, " The fact is that a large part of the admiration that has been expressed for this [Abbe] condenser has resulted, not from a comparison of its results with those of other high class achromatic condensers, but of images obtained without any sub-stage optical arrangement at all placed in contrast with results obtained by using this condenser against the same objective when used without its aid." All of these authorities speak from the standpoint of *critical* resolution of difficult objects with the highest powers of the microscope. The almost universal use of the Abbe condenser for routine work in our universities, medical schools and research laboratories testifies to its value under the ordinary conditions of microscopic investigation.

Those who are interested to go deeply into the optics and theory of the microscope should consult Carpenter-Dallinger's *The Microscope and its Revelations*, Spitta's *Microscopy* and Sir A. E. Wright's *Principles of Microscopy.*

CHAPTER XVII.

DARK GROUND ILLUMINATION.

Dark ground illumination with the low-power objectives is very useful in the examination of infusoria in stagnant water, studying small insects, eggs and scales of moths and similar objects, the coarser diatoms, polycystina, sponge spicules, pollen grains, fibres of plant and animal origin, plant hairs, crystals in plant sections, cellular tissue and any other objects whose refractive indices differ from that of the medium in which they are mounted. With the oil-immersion objective, it is *invaluable* in studying living microörganisms, the Brownian movement that often must be distinguished from other movements, fresh blood, amoeboid movements, the cilia of elementary forms of plants and animals, granules in cells, the minute markings on diatoms and like structures. With it objects so minute that they cannot be made visible by other means may be seen.

Dark ground illumination dates back to an early period in the history of the microscope, but was used rather by amateurs because of the beautiful effects obtained than by practical workers on account of its utility. In very recent years, due to improvements in apparatus, this mode of illumination has come more and more into practical use.

In the various methods of producing dark ground illumination, no light that is directly transmitted through the object is allowed to reach the objective. The object on the stage should always be shaded from extraneous light. The light by which it is seen, except when reflected light from above the stage is used, is that which is refracted or diffracted and then reflected by it from very oblique rays that are allowed to reach it, but are not allowed to reach the objective. The object practically becomes the source of light by which it is seen and stands out with almost stereoscopic clearness and with most fascinating color effects.

Lighting Opaque Objects. — With low powers dark ground illumination for opaque objects may be obtained by placing a piece of dead black cloth or paper on the stage, or on the top of the sub-stage condenser lowered to its lowest point or, swinging the sub-stage condenser and mirror to one side, over the foot of the microscope, and illuminating by light thrown onto the object from above as nearly vertically as possible. The light may be thrown onto the object by swinging the mirror above the stage, if the construction of the microscope allows this to be done. Most modern microscopes do not permit of this. The alternative is to use a bull's-eye, placed plane side towards the light, adjusting the bull's-eye and lamp so as to throw a brilliant spot of light just large enough to cover the object. It is best not to use a cover-glass, as reflections from the cover interfere with the effects. This is especially true when using this mode of illumination for photomicrography, as the effects of the reflections are much more evident in the photograph than they are visually. Apparatus of many different forms, some very complicated and expensive, has within the past two decades been contrived to use this mode of lighting for the examination of surfaces, especially those of metals in the steel and other metal industries, and for photographing such surfaces. The advance in the last few years in the practical use of the microscope for industrial purposes has been very great, and this mode of lighting is found indispensable in many of its applications. It does not come within the scope of this book to detail the different special forms of apparatus[1] and processes that the necessities of business investigations have called into existence, but mention may be made of two simple means of directing the light onto the specimen from above.

The vertical illuminator is an adjustable reflector — a totally reflecting prism or a thin disk of glass arranged at an angle of 45° with the optic axis of the microscope — enclosed in a short tube which screws into the microscope tube in place of the objective, the objective screwing into the short tube below the reflector. In the simple form shown in Fig. 76, the reflector may be turned by a milled head so that exact vertical light or more or less oblique light may be obtained. With this form the light, projected by a bull's-eye condenser, is directed onto the reflector through a small opening in the short tube. In an improved form, another tube at right angles with the first short tube carries a condensing lens that may be moved back and forth to focus the light on the reflector. The light source is placed at a short distance from the outer end of the horizontal tube. The reflector is mounted on a sliding carrier. Special short mount objectives, corrected to be used without a cover-glass, must be used with this form. This is important when medium-power dry objectives are used, but not when very low-power dry objectives or the oil-immersion objective are used. The Watson-Conrady condenser vertical illuminator, of English make, is similar in construction, but the horizontal tube has, besides a system

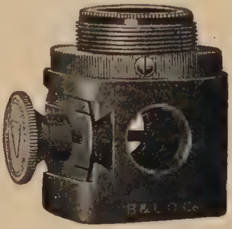

FIG. 76. — BAUSCH AND LOMB VERTICAL ILLUMINATOR.

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[1] A description of a number of special forms of such apparatus is given in Chamot's *Elementary Chemical Microscopy*, and special books on metallography deal exhaustively with them.

of lenses to concentrate the light onto the reflector, two iris diaphragms which give complete control over the light and, at the outer end of the horizontal tube, a small concentrated filament electric lamp is enclosed, making the apparatus complete in itself. Ordinary objectives are used with this type. There are several other forms of vertical illuminator, adopting the same idea in principle. In any form the light is reflected at right angles down through the objective, which acts as a condenser, onto the surface of the object placed over a black background on the stage. By the object it is reflected up through the microscope to the eye of the observer.

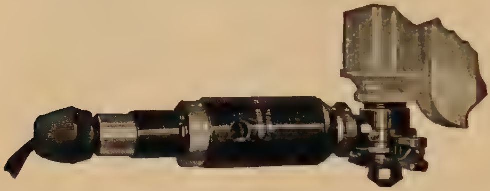

FIG. 77. — SPENCER VERTICAL ILLUMINATOR.
With side tube and attached electric lamp.

Since the last paragraph was written, the prominent manufacturers of microscopes have placed on the market modified forms of the Watson-Conrady vertical illuminator. For an example, see Fig. 77.

The Silverman illuminator is a little tungsten filament electric lamp of nearly circular form that clamps around the objective and throws the light in very nearly the vertical direction onto the object. It is easily placed in position or removed and is an efficient means of lighting opaque objects. It is expensive.

Metals and alloys are prepared for examination by reflected light by grinding and then polishing the surface until no marks of the grinding show. The surface is then usually etched, i.e., treated with some reagent that acts more on some of its component parts than on others. Then, when adjusted so that its surface is at right angles to the optic axis of the microscope, it is ready for examination.

There are many etching reagents, and one must be chosen to suit the composition of the particular specimen. The following are of somewhat general application: 100 parts absolute alcohol and 1 part of hydrochloric acid; 3 parts dilute hydrochloric acid and 1 part dilute nitric acid, 2 or 3 drops of platinum chloride being added to each 100 c.c. of the mixture; 100 parts absolute alcohol and 4 parts nitric acid; 5 per cent solution of picric acid in absolute alcohol.

It is needless to say that a considerable knowledge of the composition of metals and a large experience in examining them under the microscope are necessary for the interpretation of the results. The use of the microscope in the metal industries has come to be almost a profession by itself. Those interested should consult special books on metallography.

Lighting opaque objects by light reflected from above the stage has a very important value in photographing metal surfaces. The use of photography for this and other practical purposes is rapidly developing. Certain difficulties are met with in using a vertical illuminator. First, reflections between the object and objective and from the inner surfaces of the objective, which blur or fog the negative, are troublesome. No cover-glass should be used, as the reflections are greatly increased by it. Dry objectives should therefore be corrected to be used without a cover-glass. With an oil-immersion objective the first mentioned reflections do not occur on account of the homogeneous connection. Short mount objectives are used to bring the back lens as close as possible to the reflector of the illuminator, and absolute control over the light by means of one or more iris diaphragms is necessary to minimize the reflections.

Critical illumination (see page 283) must be secured by adjusting the source of light and condensing lenses so that the image of the light source is cast onto the object when it is in focus.

The second trouble arises from difficulties in focussing. Unless a type of vertical illuminator like the Watson-Conrady pattern, which carries the light source as an integral part of itself, is used, any change in the position of the objective occasioned by focussing in the usual way changes the relation between the light and the object. This also occurs, of course, when a vertical illuminator is used for visual observation with a microscope of the usual construction. This trouble is met in microscopes designed for metallurgical use by fitting a coarse adjustment to the stage, which is constructed to move up and down. The rough focussing is thus effected, not by moving the objective, but by moving the stage carrying the object towards or away from the objective. The final delicate focussing may be done with the ordinary fine adjustment, as the movements are so slight that they do not appreciably disturb the relations.

With the ordinary type of microscope two methods are available to overcome the difficulties in focussing. By the first method, the object is placed, not on the microscope stage, but on an auxiliary stage that fits into the sub-stage ring in place of the sub-stage condenser. The object placed on this stage is moved towards or away from the objective by means of the rack and pinion or quick screw of the sub-stage fitting. Such an auxiliary stage is easily improvised from wood. It is best made with a well-like depression at the centre. This well is filled with some plastic substance like plasticine or putty, and the object is held in this, the polished surface being made perpendicular to the optic axis of the miscroscope by raising it until it presses against a glass slip held firmly on the microscope stage. The second method, adapted to the use of the microscope in the horizontal position, is to attach at one side of the vertical illuminator a totally reflecting prism or a mirror set at an angle of 45° with the optic axis. The illuminating system, moved to one side of the optic axis of the microscope, is so placed as to direct the illuminating beam in a line parallel to the optic axis. Reflected by the prism or mirror the beam enters the vertical illuminator at right angles to the optic axis of the microscope. No matter how much the position of the objective is changed in focussing, the lighting is constant.

The difficulty in focussing is overcome in very expensive equipments by the use of a special form of inverted microscope, the objective being arranged at right angles to the body tube of the microscope and pointing vertically up. The stage, which is provided with both coarse and fine adjustments, is placed above the objective, and the specimen is placed on this with polished surface down and perpendicular to the optic axis without any adjustment.

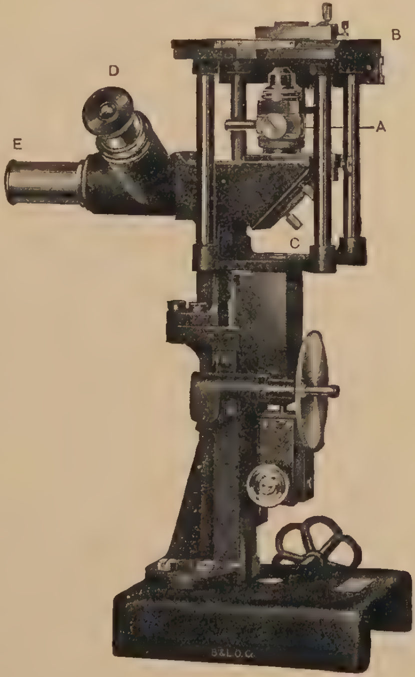

Fig. 78. — Bausch and Lomb Inverted Microscope.

Instruments of this type, made by several different manufacturers, are adapted for both visual observation and for photomicrography. For a full description, the reader is referred to books on metallography or the catalogues of the makers.

Fig. 78 shows a type of inverted microscope made by the Bausch and Lomb Optical Company. The light coming from the direction of *A* enters a vertical illuminator of either the thin glass plate or totally reflecting prism type and by it is reflected vertically upward through a short mount objective to the specimen laid on the stage *B*. By the specimen the light is reflected vertically downward until it strikes a 45° reflector at *C*, by which it is reflected horizontally to the left until it strikes another 45° reflector by which it is reflected to the eye of the observer at *D*. By pulling out the tube *D*, the light has unobstructed passage through the tube *E* which connects with the camera.

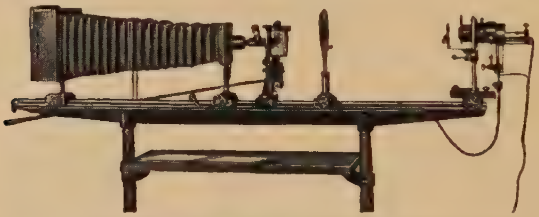

FIG. 79. — BAUSCH AND LOMB INVERTED MICROSCOPE AND CAMERA.

Arranged for metallographic work.

Unless one has apparatus suited to the purpose, it is best not to undertake to photograph with a vertical illuminator. The difficulties to be overcome are those of the proper illumination of the surface to be photographed, and without suitable apparatus these are practically insurmountable. With correct arrangements for the illumination of the specimen, the photographic difficulties are no greater than in photographing biological and histological specimens.

Opaque surfaces may be photographed by light projected on to them by a bull's-eye, but, of course, shadow effects are produced in proportion to the obliquity of the light and the roughness of the surface being photographed. If the surface is illuminated by two bull's-eyes placed on opposite sides of the microscope, the shadow effects are lessened. The Silverman reflector should give a good lighting.

Transmitted Oblique Light. — With low powers and with no sub-stage or with the sub-stage condenser swung to one side, dark ground illumination with transmitted light is imperfectly obtained by swinging the mirror far to one side and illuminating with very oblique light.

With low powers and a sub-stage condenser, it is to a certain extent obtained by swinging the mirror to one side so as to use oblique light. If the sub-stage condenser has an arrangement for decentring the iris diaphragm, it is obtained by moving the iris, well closed down, to one side of the optic axis. It is also obtained by holding the finger or a card beneath the condenser so as to shut off a part of the light, or by inserting in the ring beneath the condenser a circular piece of blackened cardboard from the edge of which a small portion has been cut so as to admit light only at one side of the condenser. Several such pieces are prepared, each with a different shaped or different sized portion cut out, or with a small circle at some distance from the centre cut out, and are tried one after another until the one that gives the best effect for the object under examination is found. Or, it may be obtained by inserting a blackened circle of cardboard three fourths to seven eighths of the distance across the lower lens of the condenser, leaving a crescent-shaped passage for the light.

Transmitted oblique light obtained as stated is an important means of studying objects with minute recurring structure such as that of diatoms. It has in recent years been much employed in studying many classes of objects for commercial purposes, as for instance foodstuffs to detect impurities; in the examination of fibres used in the manufactures and the hairs in furs; in the chemical laboratory in the study of crystalline structure; by the mineralogist in studying the structure and cleavage planes of rock sections. It should never be depended on alone, as false conclusions may easily be formed, but after all that can be learned by the use of axial light has been found out, transmitted oblique light often reveals points of great value.

Oblique transmitted light is used in photographing objects with recurrent markings. It calls for very accurate adjustments of the light, a highly corrected, properly centred and focussed sub-stage condenser and exact arrangements of all the components of the apparatus, especially when using the oil-immersion objective.

All of the foregoing methods of obtaining oblique light give only imperfect dark ground illumination and are treated by some writers as distinctly different from the methods that follow.

Dark Ground Illumination with Central Stop in the Sub-Stage Condenser. — Dark ground illumination more properly so called is usually obtained with low-power objectives by introducing a ring diaphragm into the lower part of the condenser fitting, allowing the light to pass only through the peripheral portion. The diaphragm must be adapted to the particular objective used. A ring diaphragm made on the principle of the iris diaphragm is to be obtained from some microscope makers. This can of course be adjusted for use with different low-power objectives. When the adjustments are correct, the field is black, and the objects show brightly.

Ring diaphragms may be easily cut out of thin cardboard, blackened with India ink. The diameter of the central stop should be slightly smaller than the opening in the sub-stage condenser iris diaphragm necessary just to fill the back lens of the objective. Spitta, *Photomicrography*, page 95, gives the most practical method of determining the size. Focus on some object, adjust the condenser to secure critical illumination, then lower the condenser just enough to light the whole field evenly and withdraw the object from the field of view. Remove the eye-piece, look down on the back lens of the objective, close the iris diaphragm and then slowly open it until its image, seen in the back lens, is on the point of disappearing. "Measure the aperture of the iris directly with compasses, and make the stop for the lens under consideration 7/10 of that diameter. If this little stop be suitably supported on thin arms and placed just above, or resting on the edge of the iris itself, it will be found to leave an outside ring of annular light about enough to fill the outer zone of the objective." To test its size, open the iris wide, place the stop in position in the condenser, restore the eye-piece and place on a slide a few particles of any finely powdered dry substance. If the stop is of the right size, the particles will, when viewed through the microscope, shine brightly on a dark field. If the field is gray, the stop is not sufficiently black, or the light employed is not sufficiently intense. Make the ring around the black centre as wide as possible. The higher the power of the objective, the greater is its numerical aperture, and the larger must be the diameter of the central stop, and consequently the narrower will be the ring of light admitted around it. The higher the power of the objective, therefore,

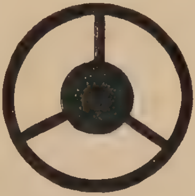
FIG. 80. — RING DIAPHRAGM.

the greater the intensity of the light necessary for good effects.

The iris diaphragm of the sub-stage condenser must usually be partly closed down when using low-power objectives to adjust its numerical aperture to the numerical aperture of the objective. The most perfect results are secured by a very delicate adjustment of these numerical apertures. To make this delicate adjustment, a diaphragm, — called a Davis nose-piece iris diaphragm, — mounted to be screwed into the nose-piece with the objective screwed into the lower end of the diaphragm fitting, is sometimes employed to control the numerical aperture of the objective. This diaphragm and the diaphragm of the sub-stage condenser are manipulated, the focussing of the condenser being frequently tested, until the effects are judged to be the best attainable. Sufficiently perfect effects for ordinary visual work are secured without the nose-piece diaphragm. It is not a part of the regular equipment, and its most important use is in photomicrography.

With a suitable ring diaphragm and an intense light, dark ground illumination may be obtained with dry objectives up to the 3 or 4 mm., if a sub-stage condenser giving a sufficiently large aplanatic cone of light is used. There should be immersion connection between the condenser and the slide.

The more highly corrected the sub-stage condenser, the more perfect are the dark ground effects produced. The Abbe condenser works well with objectives of a numerical aperture less than .5, but not well with those of a higher numerical aperture. It is the excess of the aplanatic numerical aperture of the sub-stage condenser over the numerical aperture of the objective that provides the light properly to illuminate the object.

Care must be exercised in interpreting what is seen under dark ground illumination. False images may be formed, and an entirely erroneous idea of the structure of the object may be obtained. At best it should be used only to supplement the study of objects with a full cone of light, and it is often a valuable help for this purpose.

For photomicrography with a ring diaphragm with low powers, the vertical camera is to be preferred, as the adjustments are more easily made, and the ring diaphragm is less liable to displacement. The exposures are, of course, much longer than when full illumination is employed. Lighting with a hollow cone has for general photomicrography little value except to obtain photographs of an unusual and striking appearance. The structure is always more or less obscured, and little of value is gained for the interpretation of details. No more serious difficulties are met than when using a full cone of light. In developing care must be taken to keep the plate rocking throughout the full time of development, as deposits from the developer may be made on the gelatine film, giving rise to grayness in the background of the negative, which, when a print is made, interfere with securing a uniform dead black.

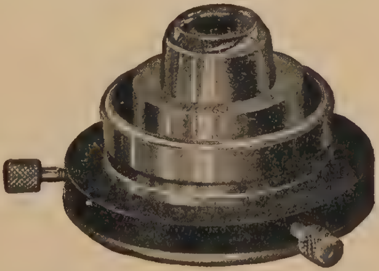

FIG. 81. — SPENCER DARK FIELD ILLUMINATOR.

Dark Ground Illumination with a Special Sub-Stage Illuminator. — Various forms of dark ground illuminator are made for use with the high-power objectives. It is beyond the writer's purpose to discuss the different types. The following description applies to the forms made by the foremost American microscope makers. A special dark ground illuminator, which is inserted in the sub-stage condenser fitting in place of the usual sub-stage condenser, must be used. This illuminator is so constructed that the central cone of light up to a numerical aperture of nearly 1 is blocked out. The light passing through the periphery is between N.A. .8 or .9 [1] and the full numerical aperture of the illuminator. As oil-immersion objectives have a numerical aperture greater than .8, some light from the dark ground illuminator could enter them. Therefore a diaphragm that comes with the illuminator must be inserted into the oil-immersion objective just above the back lens to reduce its numerical aperture to slightly less than this, so that no direct transmitted light can pass.

The microscope slide must be *scrupulously* clean, free from defects, and of a thickness suited to the focus of the illuminator, from 1 to 2 mm., varying with the different makes of illuminator. With a thicker slide the illuminator cannot be made to focus on the object, as its working distance has to be very short to make the rays transmitted by it oblique enough not to enter the objective. The cover-glass should be of the thickness for which the objectives are corrected, and this thickness should be used even with the oil-immersion objective. Especial care should be taken to see that the cover-glass is *absolutely* clean and without scratches. The layer of mounting medium between the cover-glass and slide must be as thin as possible. If too thick, particles in it and more or less out of focus of the illuminator interfere with the results. Immersion oil must be used between the dark ground illuminator and the slide, for without it light only up to the critical angle of glass and air can pass out of the dark ground illuminator, — i.e., up to 41° on each side of lines parallel with the optic axis of the microscope, —

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[1] See an article by Prof. Simon H. Gage in the 1920 volume of the *Transactions of the American Microscopical Society.*

i.e., up to N.A. .6578 (see page 285), which in this case would mean that no light could pass, as all light up to a numerical aperture of nearly 1 is blocked out by the construction. It is the light between N.A. .8 and N.A. 1.40 that is made use of, and this can be secured only by giving the light a homogeneous path to the object on the slide.

A very strong light — as the electric arc or a nitrogen filled Mazda lamp of say 400 watts — should be used, the light being projected in approximately parallel rays by a bull's-eye, placed plane side towards the light, onto the plane mirror and by that onto the illuminator. The bull's-eye and lamp should be so placed that a circle of light is projected onto the mirror of about half the diameter of the mirror. The best arrangement for lamp and lens is to combine them into a single piece of apparatus, as is done in the case of the electric lamps shown in Figs. 56 and 57 and in the case of the illuminator shown in Fig. 59. The tungsarc or 108-watt Mazda light is sufficient for a considerable percentage of work with the dark ground illuminator, but neither can take the place of the electric arc used on the direct current for work requiring the most intense illumination; on the alternating current, the electric arc is very unsatisfactory on account of fluctuations, and even when used with the direct current necessitates the interruption of the work at frequent intervals to keep the light properly centred. No such difficulties are encountered with lamps of the incandescent type. Whatever lamp is employed must be enclosed in a hood. The arrangement should be such as to shut out all extraneous light as much as possible, and the observations should be made in a darkened room.

The dark ground illuminator must be very accurately centred on the optic axis of the microscope. A study of the methods of centring for photomicrography and for the substage condenser already discussed will be helpful in this connection. The dark ground illuminator or the sub-stage ring in which it is mounted *must* have centring screws. Even greater delicacy in centring is necessary than that already discussed for a highly corrected achromatic sub-stage condenser. To centre the illuminator use a 16 mm. objective and low-power eye-piece and focus on the upper surface of the illuminator. It will help to place a *few* particles of any finely powdered substance on this surface. When these are brought into focus with the 16 mm. objective, a small circle engraved on the upper surface of the illuminator will be seen. With the centring screws bring this to the exact centre of the field of view.

The dark ground illuminator must be focussed onto the plane just above the surface of the slide bearing the objects to be examined. Slight departures from the correct centring or in the focussing may result in total failure to secure the proper illumination.

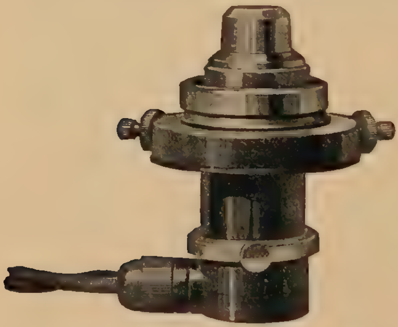

Fig. 82. — Spencer Dark Field Illuminator.

With attached lens and lamp.

A general impression of what is on the slide should be gained by using a 3 or 4 mm. objective and low-power eye-piece before using the oil-immersion objective, as it is easier to determine that the adjustments are correct with these rather than with the higher power. The proper adjustment of the mirror presents difficulties, and, if the field is not black with bright shining objects in it, the mirror should be readjusted. If changes in the position of the mirror do not give the proper results, change the size of the circle of light projected onto the mirror by the bulls'-eye, or change the relative positions of the light and mirror. Also test the focussing for each of these changes. It requires a very high degree of manipulative skill to make efficient use of this method of illumination. All adjustments and all helps to perfect illumination are taken into account.

Very recently the manufacturers have offered these illuminators with a 6-volt lamp and a lens enclosed and attached as one piece of apparatus. This arrangement simplifies the use very much and is very satisfactory for many purposes, but the light is of too low intensity for the most exacting work. See Fig. 82.

Great care must be taken in interpreting the results obtained by this method of illumination to guard against the conclusion that diffraction figures indicate the actual structure of the object. There should be much study of different objects and of the varying effects of structure on these diffraction figures before reliable conclusions can be arrived at.

With the high-power dark ground illuminator, microorganisms in the living condition have been photographed on a black background. This work requires a very high degree of skill. Sunlight or a powerful electric arc and extremely rapid plates must be used so that the exposures may be instantaneous for motile forms.

The Ultra-Microscope. — In the ultra-microscope a further extension of the principle of dark ground illumination is made. It requires special apparatus, has a very narrow range in application, and is beyond the province of any except very specialized workers. An adjustable slit like that used on a spectroscope is arranged with a special condenser at the level of the slide at right angles to the optic axis of the microscope. A very powerful light — that from the sun or an electric arc — is focussed through the condenser and slit onto the object, which is held in different ways in different types of apparatus. No light except that which is reflected or refracted by the object is allowed to reach the objective. This is in brief the principle. Numerous complicated forms of apparatus have been invented, which it is beyond the province of this book to discuss. Mention may be made of one interesting form, the Zsigmondy immersion ultra-microscope,[1] in which the light is thrown through a second high-power objective arranged at right angles to the objective on the microscope. There is no stage. No slide and no cover-glass are used. The liquid under examination is applied to the two objectives where they nearly meet and is held in position by capillary attraction. Only liquids can be examined with this and several other types of apparatus.

Particles of matter so minute that they are beyond the range of vision by any other method of lighting become visible by this method, — hence the name, the ultra-microscope. This mode of lighting simply renders the particles visible; it does not help in the study of details of the object. Its principal use is in the examination of the diffraction disks formed by exceedingly minute particles held in suspension in liquids, gases and colloid substances and in the examination of different kinds of glass and similar substances.

When this mode of illumination was discovered, extravagant claims were made, and great hopes were entertained that it would prove a means of rendering visible the ultimate constituents of matter. These hopes have been far from realized. The ultra-microscope appears to be useful only to physicists in the most recondite study of matter. The results thus far attained have been disappointing, but no one can prophesy what developments may come from its use.

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[1] This and a number of other forms of the ultra-microscope are figured and described in Chamot's *Elementary Chemical Microscopy.*

APPENDIX.

1. Grenacher's Borax Carmine.

Water	50 c.c.
Borax	2 grammes
Carmine	1 to 1½ grammes

Heat until the carmine dissolves. Add an equal volume of 70 per cent alcohol, and let it stand 24 hours. Filter.

Objects should be soaked in 35 per cent alcohol before staining and washed in same after staining. Excess of stain is removed by acid alcohol (alcohol, 35 per cent, 100 c.c.; hydrochloric acid, 4 or 5 drops). This is a good fluid for staining objects in mass.

2. Baumgarten's Borax Picro-Carmine.

This is prepared by adding picric acid crystals slowly to Grenacher's Borax Carmine until it takes on a bright blood-red color. Objects are soaked in 35 per cent alcohol before staining, and excess of stain is washed out in same.

3. Grenacher's Alum Carmine.

Water	50 c.c.
Alum (ammonia or potash)	1 to 3 grammes
Carmine	½ gramme

Boil 15 to 20 minutes, cool, and filter. Add a drop or two of carbolic acid to preserve it.

Objects should be soaked in water before staining, and washed in same after staining. Excess of stain is removed by water.

4. Beale's Carmine.

Ammonia	1 c.c.
Water	20 c.c.
Carmine	½ gramme

When the carmine is dissolved, add 28 c.c. water, and let it stand open to the air, but protected from dust, until the odor of ammonia is faint. Then add 48 c.c. glycerine and 12 c.c. alcohol.

In using, pour a little of the fluid into an open dish with an equal volume of glycerine, and mix thoroughly. The action is slow, requiring many hours. An old fluid is quicker in action than a fresh one.

Objects should be soaked in water before staining, washed in same after staining, then passed through very dilute hydrochloric acid and again washed in water.

Excess of stain is removed by passing object quickly through very dilute ammonia (1 drop ammonia to 5 c.c. water), and then washing thoroughly in water.

5. Gerlach's Ammonia Carmine.

Water	30 c.c.
Carmine	.2 to .4 grammes
Ammonia	A few drops

Mix and, after the most of the carmine has dissolved, filter. Allow it to stand exposed to the air, but protected from dust, until the odor of ammonia is faint. If the carmine separates from the solution, add a small drop of ammonia. For use, add this fluid, drop by drop, to water until the right shade is obtained.

Objects should be soaked in water before staining, and washed in water after staining. Excess of stain is removed by passing quickly through very dilute ammonia.

6. Weigert's Picro-Carmine.

Saturated solution of picric acid	100 c.c.
Ammonia	2 c.c.
Carmine	1 gramme

Let the carmine stand in the ammonia for 24 hours, add the picric acid solution, and let it stand another 24 hours. Add acetic acid drop by drop until a slight precipitate persists. Let it stand another 24 hours and filter. The precipitate will not all be removed by filtering. Add ammonia a drop at a time at intervals of several hours until the precipitate dissolves and the solution becomes perfectly clear. If the yellow stain due to the picric acid is too prominent, it may be diminished by adding minute quantities of acetic acid. If the red from the carmine is too prominent, it may be corrected by adding ammonia. Objects should be soaked in water before staining and washed in water after staining.

7. Löwenthal's Picro-Carmine.

One per cent solution of caustic soda .	100 c.c.
Carmine	.4 gramme
Water	100 c.c.
One per cent aqueous picric acid . . .	25 c.c.

Dissolve the carmine in the caustic soda solution, filter, and add the water. Then pour in the picric acid solution slowly. This will produce a precipitate which will in time nearly but not quite all dissolve. If a slight precipitate does not persist, add a little more of the picric acid solution, and filter several times. Object should be soaked in water before staining and washed out in water after staining.

8. Orth's Lithium Carmine.

Saturated aqueous solution of lithium carbonate .	100 c.c.
Carmine	2 grammes

Sections may be passed from water into this stain. Excess of stain may be removed by washing in acid alcohol (70 per cent alcohol, 100 c.c.; hydrochloric acid, 1 c.c.). Thus used, this is a good nuclear stain. Without the washing in acid alcohol, the stain is quite diffuse. This stain is highly recommended. Good double staining results are obtained by following this stain with an aqueous solution of picric acid. Sections stained in lithium carmine cannot be fixed to the slide with albumen fixative.

9. Mayer's Cochineal Tincture.

Alcohol, 70 per cent	10 c.c.
Powdered cochineal	1 gramme

Mix, let it stand some days, and filter. If the action is too intense, dilute with 70 per cent alcohol.

Objects should be soaked in 70 per cent alcohol before staining, and washed in same after staining.

10. Kleinenberg's Hæmatoxylin.

a. To a saturated solution of calcium chloride in 70 per cent alcohol add an excess of pure alum, and let it stand 24 hours; filter, and add 8 volumes of 70 per cent alcohol. If necessary, filter again.

b. Dissolve crystallized hæmatoxylin in absolute alcohol to saturation. Add this to the first solution until the right intensity of color is obtained. The fluid thus obtained may be diluted at any time by further addition of the first solution. Object should be soaked in 70 per cent alcohol before staining, and washed in same after staining. Object must contain no acid, or the color will fade.

Excess of stain is removed with acidulated alcohol (70 per cent alcohol, 100 c.c.; hydrochloric acid, ½ c.c.). Wash thoroughly again in 70 per cent alcohol to remove acid.

An old solution is better than a fresh one.

11. Grenacher's Hæmatoxylin.

Mix 4 c.c. of a saturated solution of hæmatoxylin crystals in absolute alcohol with 150 c.c. of a saturated solution of ammonia alum in water, and let the mixture stand exposed to the light for a week. Filter, and add 25 c.c. of glycerine and 25 c.c. of methyl alcohol (wood spirit). An old solution is better than a fresh one.

Object should be placed in water before and after staining.

12. Böhmer's Hæmatoxylin.

a. Dissolve .35 gramme of hæmatoxylin in 10 c.c. absolute alcohol.

b. Dissolve 2 grammes of alum in 100 c.c. of water.

c. Add solution *a* to solution *b* until a bright violet solution is obtained and allow the resulting solution to ripen, protected from the dust but exposed to the air, for two or three weeks before using. Object should be treated with water before and after staining. This is a good stain for sections of objects that have been fixed in corrosive sublimate, alcohol, picric acid, and nitric acid. The sections stain well after they are fixed on the slide with Mayer's albumen fixative.

13. Heidenhain's Hæmatoxylin.

This is prepared by simply dissolving .33 gramme of hæmatoxylin in 100 c.c. of water. This is a good stain for objects in the mass that have been fixed in picric acid or alcohol, or in chromic acid well washed out. The objects are passed from water into the coloring solution where they remain 24 hours; they are then placed in an aqueous solution of neutral potassium chromate for 24 hours, changing the solution several times. Wash in water and pass through the alcohols of gradually increasing strength.

14. Aniline Stains.

The aniline dyes give more satisfactory results when used in alcoholic solution, though they may be used in watery solution. Make saturated solutions in 95 per cent alcohol of fuchsin, Bismarck brown, iodine green, methyl green, methyl violet, and eosin. Filter, and keep as stock solutions to be diluted with 95 per cent alcohol or water to any desired degree. These solutions do not keep very well. Small quantities, therefore, should be prepared at a time. Excess of stain should be discharged in 95 per cent alcohol. The specimen should be cleared in benzole or oil of cedar. The aniline stains are not very permanent. Objects cannot be stained in them *in toto,* with the exception of Bismarck brown.

15. Bismarck Brown.

Dissolve 1 gramme of Bismarck brown in 100 c.c. of water by boiling. Filter, and add 33 c.c. of absolute alcohol. Objects may pass from water or dilute alcohol into this stain. Wash out with water, followed with alcohol. A good nuclear stain for objects hardened in chromic acid.

16. Safranin.

Pfitzner's method of preparing the safranin is to dissolve 1 gramme of the safranin in 100 c.c. absolute alcohol. After some days add 200 c.c. of water. Sections are treated with the stain for 24 hours and then washed out in strong alcohol. This is a very fine nuclear stain for sections of objects that have been fixed in Flemming's solution. If sections which have been fixed in Flemming's solution and stained in safranin are treated with acid absolute alcohol (alcohol, 100 c.c.; hydrochloric acid, .25 to .5 c.c.), only the chromatin of the nuclei will remain colored.

Equal volumes of aniline water and a strong alcoholic solution of safranin are also highly recommended as a stain for lignified and suberized cell-walls of plants. The specimens should remain in the stain one or two hours and should then be washed out with alcohol. If this stain be followed with methyl blue in aqueous solution, a good double stain is obtained.

17. Gram's Gentian Violet.

Gentian violet	1 gramme
Alcohol	15 c.c.
Aniline oil	3 c.c.
Water	80–100 c.c.

Sections are allowed to remain in this stain from one or two minutes up to several hours according to the manner in which they have been fixed and hardened. One or two minutes is often sufficient for specimens fixed and hardened in alcohol; ten or twelve hours may not be too much for specimens treated with solutions containing chromic acid. Rinse the sections in alcohol, and treat them for two or three minutes with the following solution:

Iodine	1 gramme
Potassium iodide	2 grammes
Water	300 c.c.

Rinse again in alcohol and wash with several changes of oil of cloves. If eosin be dissolved in the oil of cloves first applied, a good double staining is obtained on many tissues. Use only oil of cloves for clearing in this process. This stain is highly recommended for isolated bacteria and for nuclei.

18. Dahlia.

An aqueous solution stains the protoplasm blue or violet, leaving the nuclei without much color. Subsequent treatment with dilute acetic acid removes the color from the protoplasm and gives a blue-violet color to the nuclei. The stain should act for some hours and then be washed out with alcohol.

19. Double Stains.

For double staining the following combinations are recommended: Carmine-hæmatoxylin, carmine-dahlia, carmine-picric acid, hæmatoxylin-eosin, hæmatoxylin-picric acid, hæmatoxylin-safranin, safranin-methyl blue, safranin-gentian violet, safranin-methyl green, safranin-picric acid, methyl green-eosin, methyl green-fuchsin, fuchsin-Bismarck brown, picro-carmine-methyl green. The carmine and hæmatoxylin solutions are used according to the directions already given. The treatment with the aniline stains should, as a rule, follow the treatment with carmine or hæmatoxylin. The aniline stains should be allowed to act for some time, giving an intense coloration which is washed out in alcohol.

20. Fuchsin — Opal Blue. (Double Stain.)

a. Dissolve .1 gramme fuchsin in 44 c.c. 95 per cent alcohol.
b. Dissolve .1 gramme opal blue in 30 c.c. 95 per cent alcohol.
c. For use, mix equal parts of *a* and *b*.

21. Fuchsin — Iodine Green. (Double Stain.)

a. Dissolve fuchsin in 50 per cent alcohol.
b. Dissolve iodine green in 50 per cent alcohol.
c. Add *a* to *b*, drop by drop, until the color is violet.

22. Schällibaum's Collodion-Fixative.

Ether-collodion	1 part
Oil of cloves	3 parts

If this is found not to work well, increase the proportion of collodion. Place a thin coat of this on the centre of a dry slide; place the section on this, and press it flat with a piece of oiled paper; place the slide in the water-bath until the oil of cloves has evaporated. If these details have been properly attended to, the section will be firmly fastened to the slide. The slide may be passed through successive solutions, and the section will not be loosened. If too much of the fixative is used, there will be a slight tendency to cloudiness. The fixative does not take borax-carmine stain, but does take hæmatoxylin to a small extent. This fixative is more highly recommended for objects that have been stained in the mass than for those to be stained on the slide.

23. Mayer's Albumen Fixative.

White of egg	50 c.c.
Glycerine	50 c.c.
Sodium salicylate	1 gramme

This fixative is highly recommended for fixing sections to be stained on the slide, and especially for those to be stained in the anilines. A little of the fixative is applied to the slide with a brush, the section is placed in position, and the slide is placed in a water-bath until the paraffin melts. Remove the paraffin with turpentine, wash in alcohol thoroughly to remove the last traces of glycerine. These operations are best done by immersing the slide bearing the section in vessels of turpentine and alcohol, giving time enough for thorough action. Then clear and mount in balsam.

24. Gelatine Fixative.

Dissolve 5 grammes of gelatine in 30 c.c. of 50 per cent acetic acid. Then add 5 c.c. of 95 per cent alcohol and 1 c.c. of glycerine.

This solution is used to fix diatoms, scales of insects, and other similar objects to the cover-glass or glass slip. Apply the fixative with a brush and let it dry. After the objects are arranged, breathe carefully on it. The moisture of the breath dissolves the gelatine sufficiently to allow the objects to be stuck to it after it has again hardened.

25. Transparent Cements.

The cement used in closing mounts should in every case be transparent. When the mount has been securely closed, and the transparent cement seasoned, colored cement may be applied. Reliable cements may be obtained ready prepared of the dealers in microscopical supplies, and it is perhaps better to obtain them thus than to attempt to prepare them by the aid of the various recipes that others have used.

Shellac cement may be made as follows: Make a saturated solution of bleached shellac in 95 per cent alcohol. Let this solution stand protected from the dust until, by evaporation of the alcohol, it is reduced to the consistency of a thick syrup. Add a little turpentine to render the cement less brittle. Much of the bleached shellac on the market is artificial, and is worthless for this purpose. Only real shellac should be used. Shellac dissolves very slowly in alcohol. The bottle should be allowed to stand some days, and should be occasionally shaken.

In applying the cement, keep the brush clean by frequently washing it in 95 per cent alcohol. If the cement becomes too thick, it may be thinned with 95 per cent alcohol. This cement is especially useful for mounts which are to be examined under immersion lenses, as the cedar oil of the immersion fluid does not act upon it, as is the case with most cements. If the mounts have already been ringed with other cements, apply a light varnish of shellac cement.

Berry's hard oil finish, used by painters, is highly recommended as a transparent cement.

26. Colored Cements.

Colored cements may be made by adding to the transparent shellac cement sufficient of a filtered saturated solution in 95 per cent alcohol of fuchsin, iodine green, or opal blue, to give a strong color.

27. Preservative Media.

a. Alcohol of above 70 per cent strength. Best used after objects have been fixed and hardened by one of the fluids given in number 29.

b. Formalin.

Formalin	1 part
Water	70 parts

Chrome alum enough to give a deep green color.

This is an excellent preservative for green plants, causing the color to remain to a considerable extent. There is also less shrinkage than when alcohol is used.

The vapor of formalin is also used to harden the gelatine film of plate cultivations of bacteria so that the plates may be used as lantern slides.

c. Wickersheimer's fluid. The formula is :

Boiling water	1000 c.c.
Alum	33.3 grammes
Common salt	8.3 grammes
Potassium nitrate	4. grammes
Potassium carbonate	20. grammes
Arsenic trioxide	3.3 grammes

After the solution has cooled, filter, and add

Glycerine	400 c.c.
Alcohol, 95 per cent	100 c.c.

If small animals, the internal organs, muscles, etc., of larger animals are treated with this fluid for one or two weeks according to size, they may be removed and dried. In the dried condition they will keep an indefinite time, retaining much of their natural color. It does not make tissues brittle.

This fluid is also a good preservative for vegetable specimens, causing much less loss of color and much less shrinkage than does alcohol.

28. Killing Fluids.

The following fluids may serve for killing small animals:

a. Alcohol added drop by drop slowly to the water in which the animals are immersed. See page 144.

b. Corrosive sublimate (mercuric chloride) in saturated aqueous or alcoholic solution. See page 144.

c. Chromic acid in 1 to 2 per cent solution. See page 144.

d. Merkel's fluid; this consists of equal parts of a ¼ per cent solution of chromic acid and a ¼ per cent solution of platinum chloride. See pages 125 and 144.

e. Chloroform. See page 145.

f. Nicotine. See page 145.

29. Fixing and Hardening Fluids.

a. For most purposes alcohol, used as described in the preceding pages, is a good hardening fluid. Other fluids are better than alcohol for some special cases. Whatever fluid is used, the pieces of tissue to be hardened should be small, a large volume of the fluid should be used, and the fluid should be changed frequently, especially during the early part of the process. Several of the fluids already mentioned as killing fluids are also excellent fixing and hardening fluids, as:

b. Corrosive sublimate. See page 145.

c. Chromic acid. See page 144.

d. Merkel's fluid. See pages 125 and 144.

e. Picro-sulphuric acid. See pages 124 and 146.

f. Osmic acid. See pages 124 and 145.

g. Zenker's fluid. See page 146.

h. Mayer's picro-nitric acid. This is made by saturating 5 per cent nitric acid with picric acid; it is recommended for use on objects containing calcareous portions. Objects should remain in this fluid from three to twelve hours according to their size. They should then be washed in 70 per cent and the higher alcohols until the yellow color due to the picric acid has wholly disappeared. Hæmatoxylin is a good stain.

i. Flemming's chromo-aceto-osmic fluid. See page 145. This is one of the most highly recommended fluids for general hardening purposes.

j. Perenyi's fluid. The formula is:

10 per cent nitric acid	40 c.c.
Alcohol	30 c.c.
½ per cent aqueous solution of chromic acid .	30 c.c.

This fluid is especially recommended for ova. The objects should remain in it from a few minutes to six hours according to their character. They are then washed out in 70 per cent alcohol and hardened by treatment with the higher alcohols, remaining two or three days in each grade.

k. Rabl's fluid. The formula is:

⅓ per cent chromic acid	200 c.c.
Formic acid	4 or 5 drops

This fluid is recommended for bringing out nuclear figures in temporary preparations both vegetable and animal. It does not keep well and should be prepared fresh each time it is to be used. It should act about 24 hours. Wash out in water followed by alcohols of increasing strength. Stain with hæmatoxylin or safranin, or double stain with the two. Rabl also used platinum chloride (1 gramme platinum chloride to 300 c.c. of water) for the same purpose and followed with the same stains.

l. Iodine. A few drops of the tincture of iodine added to sea-water is a solution highly recommended for fixing the cell-contents of marine algæ. After the fluid has acted for one or two minutes, the algæ may be dehydrated in alcohol, beginning with 40 or 50 per cent.

m. Müller's fluid is much slower in action than alcohol, the time varying with different tissues from a few days to eight or ten weeks. The colder the fluid, the better the results. The formula is:

Distilled water	1 litre
Potassium dichromate, $K_2Cr_2O_7$ . . .	25 grammes
Sodium sulphate, Na_2SO_4	10 grammes

n. Erlicki's fluid is recommended for hardening brain tissues, which require about two weeks. The formula is: —

Distilled water	1 litre
Potassium dichromate, $K_2Cr_2O_7$ . . .	25 grammes
Copper sulphate, $CuSO_4$	5 grammes

30. Indifferent Media.

In the study of fresh tissues it is often of great importance that the fluid in which they are examined shall not produce changes in the specimens during the examination. No fluids are known that are wholly without influence on tissues. The following have been devised by different workers and are the best known media for the prevention of osmosis and for preserving tissues unchanged.

a. Normal or physiological salt solution (common salt, .75 gramme; water, 100 c.c.).

b. Sugar solution. (This consists of a nearly saturated solution of sugar in water to which one per cent of carbolic acid or one per cent of chloral hydrate has been added.)

c. The juices of fruits.

d. The white of egg.

e. Aqueous humor (obtained from the eyes of recently killed animals).

f. Saliva.

g. Blood serum. Whitman and Lee, following Ranvier and Schultze, recommend very highly the amniotic fluid of mammals to which iodine has been added. The fluid can be obtained at slaughter houses.

The first three of these are naturally selected as the media for the examination of delicate plant tissues; all are useful agents in the examination of animal tissues.

31. Macerating Fluids.

a.	Water.	
b.	Caustic potash, KOH, or caustic soda, $NaOH$	30 grammes
	Water	70 c.c.
c.	Potassium hypochlorite, $KClO$ (Eau de Javelle)	8 drops
	Water	100 c.c.
d.	Alcohol of less than 30 per cent.	
e.	Schultze's macerating mixture:	
	Potassium chlorate, $KClO_3$	1 gramme
	Nitric acid, HNO_3	50 c.c.

The last mixture may be used either hot or cold.

32. Clearing Agents.

Before objects can be successfully imbedded in paraffin, and before sections can be mounted in balsam, they must be entirely freed from water. Even the best of absolute alcohol contains some water. It is therefore necessary to interpose between the alcohol and the paraffin or balsam some medium which will drive the alcohol out of the object and which is at the same time miscible with paraffin or balsam. If delicate objects are transferred directly from alcohol to a clearing agent, a considerable amount of shrinkage and distortion usually occurs. To prevent this, it is generally better to place the object in a test-tube with a little alcohol and then introduce the clearing agent into the very bottom of the tube by means of a pipette, allowing it to flow in slowly. As soon as the object settles into the clearing agent, pour off the mixed fluids and pour in a fresh portion of the clearing agent. A great many different substances may be used. A few of those that are most generally useful are enumerated:

a. Oil of cedar. This is the best clearing agent for sections stained in aniline colors. It may follow 95 per cent alcohol, and it does not cause shrinkage of the objects. It is an excellent medium in passing from alcohol to melted paraffin and also in passing from alcohol to chloroform balsam. Prolonged treatment with this oil clears celloidin sections, but it is not recommended for this purpose.

b. Oil of origanum. This extracts aniline colors to some extent. It clears objects taken directly from 95 per cent alcohol. It is a good clearing agent for celloidin sections, but care must be taken to use the pure oil.

c. Oil of cloves. This is not reliable for objects stained in anilines. Neither is it to be recommended for use in passing from alcohol to paraffin, as it tends to make objects brittle; it also mixes very slowly with the paraffin. If it is used to free from alcohol, it is better to follow it with turpentine, before placing the object in paraffin. It is not to be used for celloidin sections, as it dissolves the celloidin. It quickly clears objects that have been treated with 95 per cent alcohol, and is a valuable clearing agent except for the classes of objects already mentioned.

d. Oil of turpentine. This clears objects taken direct from 95 per cent alcohol with a fair degree of rapidity. It has a tendency to shrink the objects, and also to render them brittle. In passing from alcohol to turpentine it is well to use first a mixture of two-thirds alcohol and one-third turpentine, then a mixture of one-third alcohol and two-thirds turpentine, finally pure turpentine.

e. Chloroform. This clears objects taken from 95 per cent alcohol. It dissolves paraffin very readily. It is therefore a good agent in passing from alcohol to paraffin. A little sulphuric ether mixed with the chloroform improves it for this use. Care must be taken to keep the objects in melted paraffin on the water-bath until all of the chloroform has been expelled. If this is not done, bubbles will form in the paraffin cast as it cools, rendering it unfit for use. Chloroform is an excellent clearing agent for sections cut in celloidin. It is important that the sections be well dehydrated before the chloroform is applied. Alcohol of 95 per cent strength will dehydrate sufficiently, but it is to be remembered that the alcohol of commerce is frequently below this strength. Resort must sometimes be had to what is known as absolute alcohol. Care must be exercised not to allow the sections to remain too long in the alcohol, or the celloidin will be dissolved. The chloroform is to be applied repeatedly until the celloidin becomes perfectly transparent. Chloroform balsam is then quickly applied, and the cover-glass is put in position. Chloroform may be used to clear sections cut in paraffin or in pith, but it is not especially recommended for these classes of objects. Use only the best chloroform.

f. Carbolic acid. This agent clears instantly. It may be used even when the object has been very imperfectly dehydrated, for it has the power to drive out a considerable amount of water. It is therefore a good clearing agent for celloidin sections. A considerable amount of shrinkage of the object is, however, liable to result. For use, the carbolic acid crystals may be dissolved in alcohol or oil of turpentine. A concentrated solution should be made in either case. The solution in turpentine is especially good.

g. Creosote. Beechwood creosote is an excellent clearing agent, especially for sections cut in celloidin.

h. Benzin, benzole, naphtha, toluol, and xylol may be mentioned as other good clearing agents. The dehydration must be very perfect before these fluids are applied. It is generally best to dehydrate in absolute alcohol, though with care 95 per cent alcohol will serve.

33. Mounting Media.

a. Canada balsam, pure or dissolved in benzole, xylol, or chloroform. It is best purchased ready prepared.

b. Glycerine.

c. Glycerine-jelly. It is best purchased ready prepared. One formula is:

Best gelatine	1 part
Water	3 parts
Glycerine	4 parts

Soak the gelatine in the water until it is soft. Add the glycerine, and heat over a water-bath. Then add two or three drops of carbolic acid as a preservative.

d. Gum arabic dissolved in water. Use for vegetable sections.

e. Gum arabic dissolved in a saturated aqueous solution of potassium acetate to the consistency of a thick syrup. (This is Hoyer's Medium for vegetable sections stained in aniline dyes.)

f. Gum arabic dissolved in a 10 per cent solution of chloral hydrate in water, and one-tenth the volume of glycerine added. (This is Hoyer's Medium for vegetable objects stained in carmine solutions.)

g. Gum arabic dissolved in turpentine, or in equal parts of turpentine and benzole. This is a substitute for Canada balsam, but is much inferior to it.

h. Farrant's Medium for either animal or vegetable objects:

Gum arabic	2 grammes
Water	4 c.c.
Glycerine	2 c.c.

Dissolve the gum arabic in the water and add the glycerine. Stir, but do not shake, to effect solution. Strain through cloth, if necessary.

i. Media for algæ and other green plants. No media are known that will perfectly preserve all delicate green plants in their natural color and without shrinkage. Some media work well with some plants but are useless with others. The following solutions are recommended:

1. Camphor water. This is simply a saturated solution of camphor in distilled water.

2. Kirchner's fluid. This is made by dissolving chrome alum in dilute glycerine until a clear blue solution is obtained.

3. Carbolic acid. 1 per cent solution in water.

4. Chloral hydrate. 5 per cent solution in water.

5. Petit's fluid:

Water saturated with camphor	50. c.c.
Water	50. c.c.
Acetic acid (glacial)	.5 gramme
Chloride of copper (crystallized)	.2 gramme
Nitrate of copper	.2 gramme

6. Goadby's fluid:

Common salt	120. grammes
Alum	60. grammes
Corrosive sublimate	.25 gramme
Water	2330. c.c.

7. Pacini's fluid:

Water	1000 c.c.
Common salt	10 grammes
Corrosive sublimate	5 grammes

8. A saturated solution of potassium acetate in water.

9. King's fluid for marine algæ:

Corrosive sublimate	.258 gramme
Alum	62.2 grammes
Sea-water	2270. c.c.

34. Solutions for Cleaning Glass Slips and Cover-Glasses.

a. Borax dissolved in water.

b. Washing soda (sodium carbonate, Na_2CO_3) dissolved in water.

c. Caustic potash (potassium hydroxide, KOH) dissolved in water.

d.

Potassium dichromate, $K_2Cr_2O_7$	10 grammes
Water	50 c.c.
Sulphuric acid, H_2SO_4	50 c.c.

35. Acids.

Sulphuric acid, H_2SO_4, hydrochloric acid, HCl, and nitric acid, HNO_3, should be used in full strength when the acid is simply mentioned. Special strengths of these acids have been mentioned for particular purposes. For ordinary use, the common commercial acids are sufficiently pure. If, however, the acids are purchased especially for microscopical work, the chemically pure acids should be obtained.

36. Alcohol of Percentage Strength.

In these pages, alcohol of a particular percentage strength has been required for many purposes. It is not always convenient to stop and compute the strength; the following table is therefore added. The strength of ordinary commercial alcohol is assumed to be 95 per cent. It should be this, but often falls short of it. The table is computed not for mathematical accuracy, but for practical utility. It is sufficiently accurate for all the purposes described in this book. A few ounces of calcium oxide (unslaked lime), calcium chloride, or copper sulphate to the gallon will dehydrate ordinary alcohol and render it practically absolute.

To Make	Use	
	95 % Alcohol.	Water.
95 % alcohol	100 parts	0 parts
90 % “	95 “	5 “
85 % “	89 “	11 “
82 % “	86 “	14 “
80 % “	84 “	16 “
75 % “	79 “	21 “
70 % “	74 “	26 “
65 % “	68 “	32 “
60 % “	63 “	37 “
50 % “	53 “	47 “
40 % “	42 “	58 “
35 % “	37 “	63 “
30 % “	32 “	68 “

37. Iodine Solutions. (Test for Starch.)

a. Iodine dissolved in water. The solution takes place very slowly.
b. Iodine dissolved in alcohol.
c. Iodine dissolved in a water solution of potassium iodide, KI.

38. Chlorzinc Iodine. (Schultze's Solution. Test for Cellulose.)

Dissolve in hydrochloric acid all the metallic zinc that will dissolve, and evaporate to the consistency of a thick syrup. Add to this solution all the potassium iodide, KI, that will dissolve. Then add all the iodine that will dissolve. Filter through glass wool.

39. Ammoniacal Cupric Oxide. (Test for Cellulose.)

Dissolve in water all the copper sulphate, $CuSO_4$, that will dissolve. Add to this solution caustic soda, $NaOH$, or caustic potash, KOH, as long as a precipitate is formed. Filter, and wash the precipitate in water that has been boiled. Place this greenish precipitate in a bottle and add just enough strong ammonia, NH_4OH, to dissolve it. To test it, see if fibres of cotton will dissolve in it. Keep in a dark place. Freshly made solutions are best.

40. Mucilage for Labels.

Dissolve 100 grammes of gum arabic in 250 c.c. of water and add 2 grammes of crystallized aluminium sulphate dissolved in 20 c.c. of water.

41. Warm Stage.

A simple but effective warm stage may be made from a strip of sheet metal six or eight inches long and three inches wide with a hole an inch in diameter at such a distance from one end as to coincide with the opening in the stage when the strip is placed on the stage, the free end projecting in front. The strip is held in position by the spring clips. The object to be examined is mounted in the usual way on a glass slip, and this is placed on the strip of metal. The free end of the strip is warmed by a Bunsen burner or an alcohol lamp. A piece of paraffin, whose melting-point is known, may be placed on the strip near the opening to serve as an indicator to prevent overheating the specimen under examination.

42. Thermometer Readings.

$\frac{5}{9}$ (degrees Fahrenheit $-32°$) = degrees Centigrade.
$\frac{9}{5}$ (degrees Centigrade $+32°$) = degrees Fahrenheit.

43. Metric-English Equivalents.

1 gramme	=	15.4 grains
1 ounce avoirdupois	=	28.3 grammes
1 ounce Troy	=	31.1 grammes
1 fluid ounce	=	29.25 c.c.
1 pint	=	16 fluid ounces

44. The Micron.

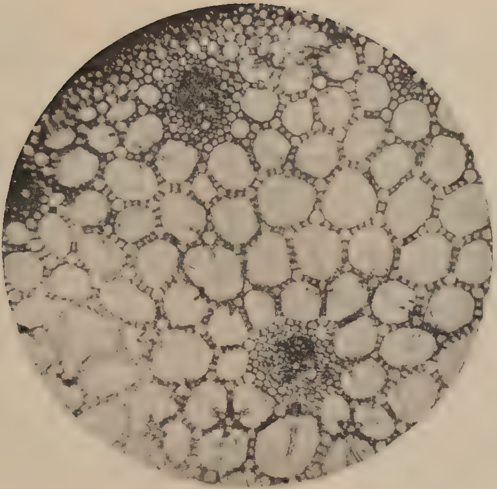
The unit for microscopical measurements is the micron, represented by the Greek letter μ. It is sometimes called the micro-millimetre.

$$1\ \mu = .001 \text{ millimeter} = .000039 \text{ inch.}$$

NOTE.

THE reproductions of photo-micrographs shown in the following plates were made by the half-tone process, a process that gives a decided dark tint to the white portions of a photograph. A good photo-micrograph should show the structure of the object clearly, and at the same time have an undarkened background. The photo-micrographs that are reproduced here have clear and brilliant whites; the dark background of the reproductions is due to the process used.

These photo-micrographs are introduced to give those who are not familiar with the work some idea of the results that may be obtained in low-power photo-micrography. Descriptions of the plates will be found in the List of Illustrations, pages xviii–xxviii.



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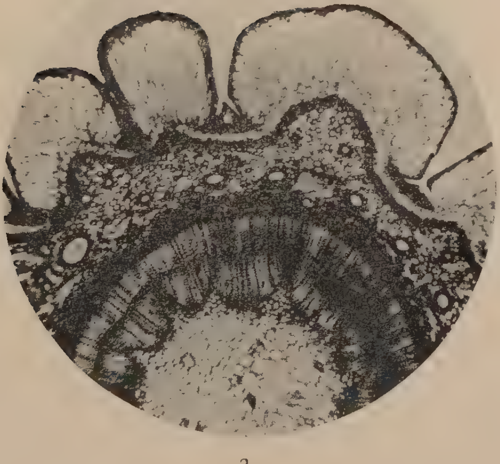

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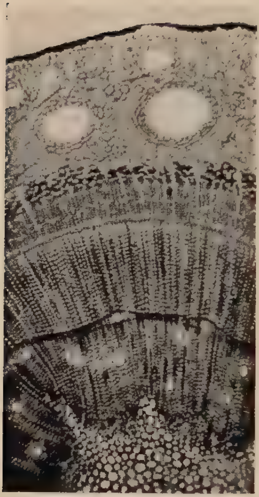

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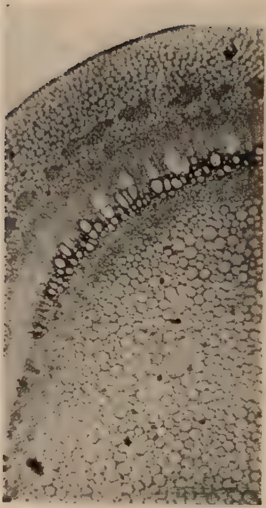

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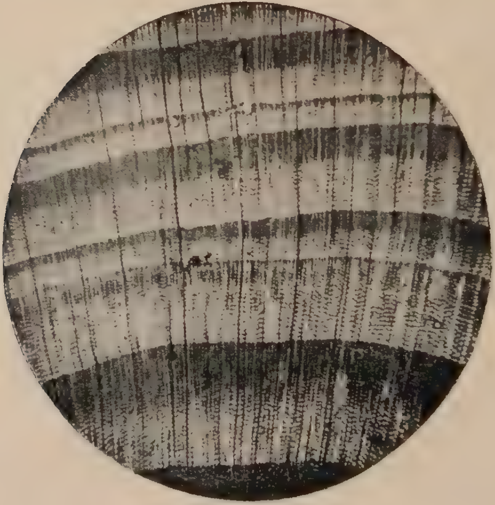

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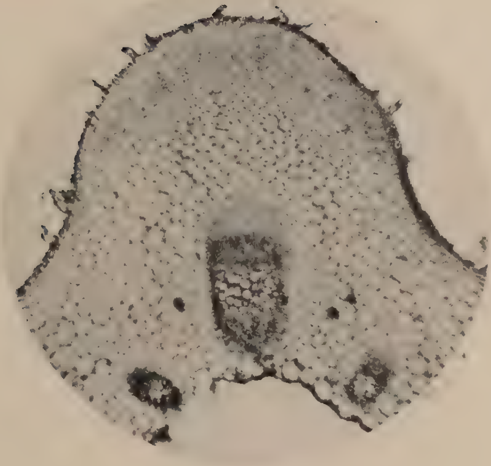

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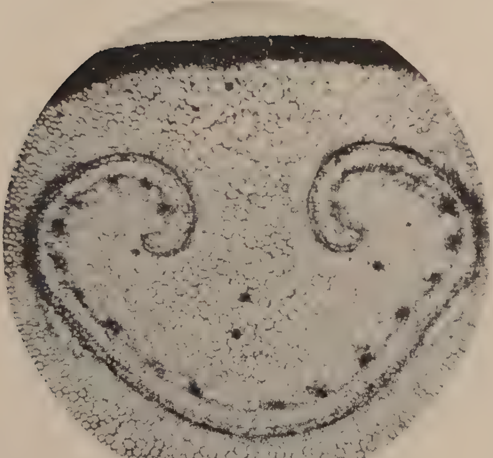

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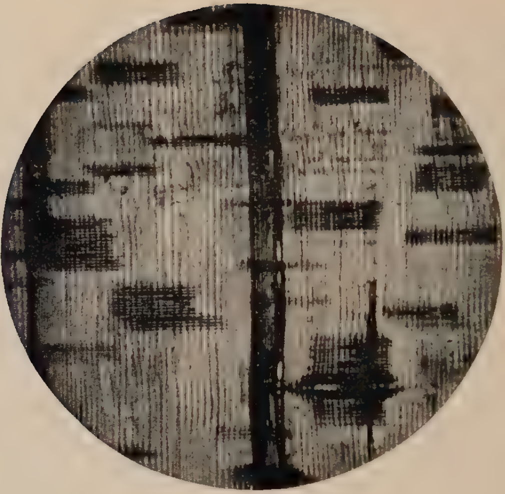

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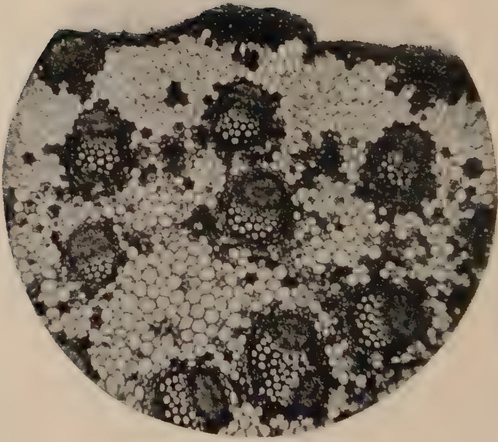

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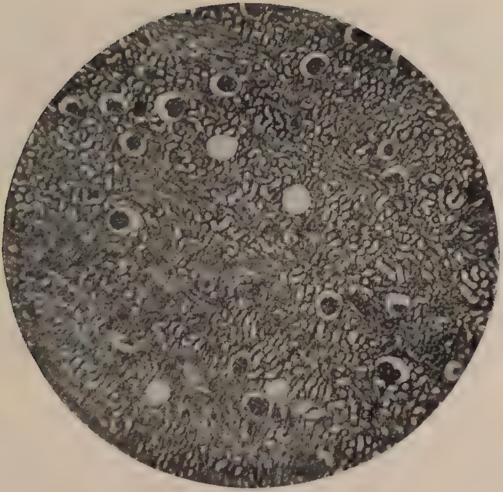

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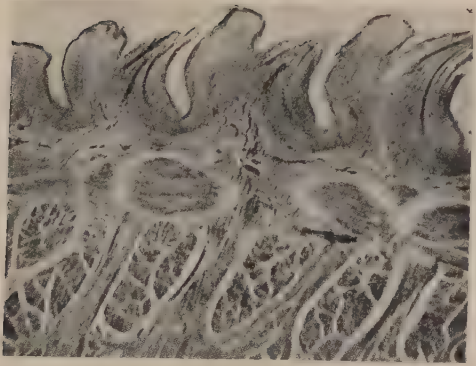

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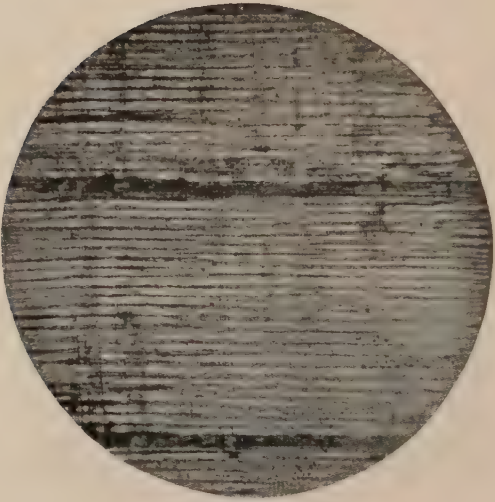

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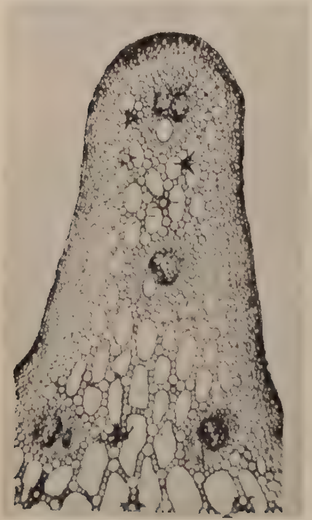

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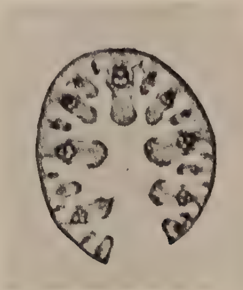

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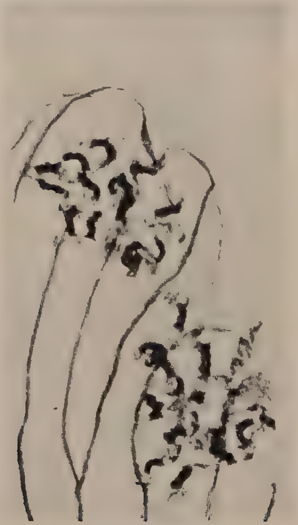

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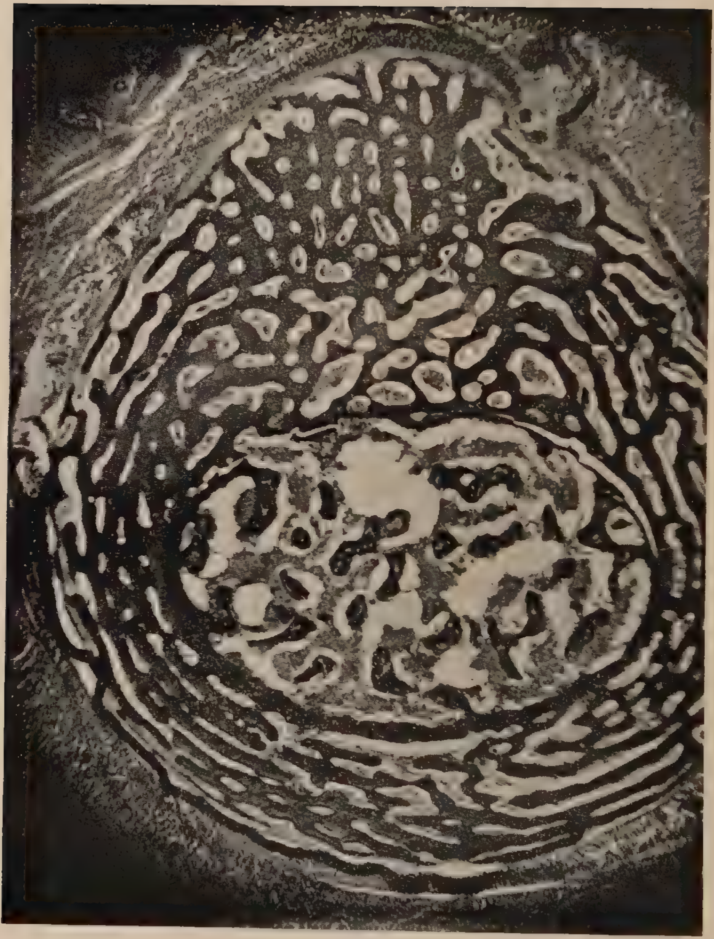

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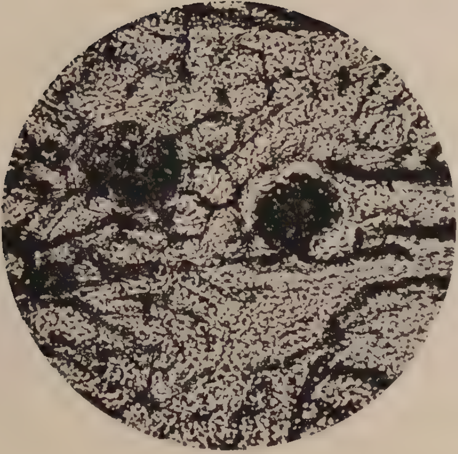

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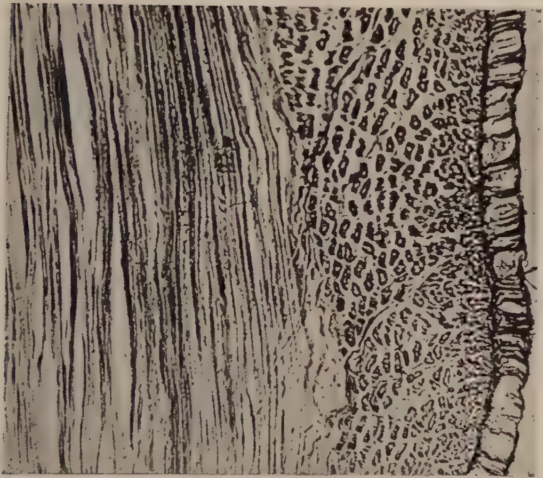

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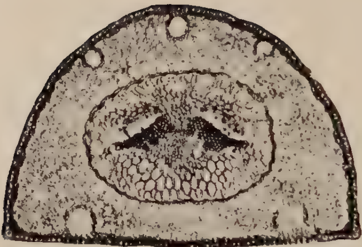

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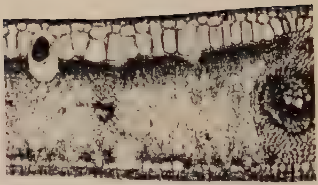

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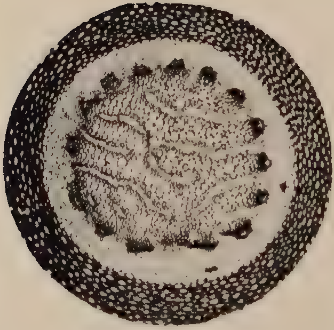

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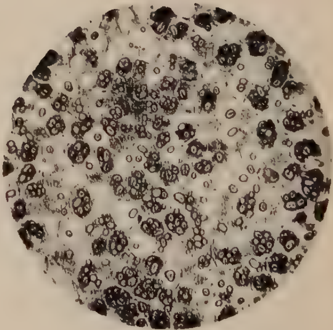

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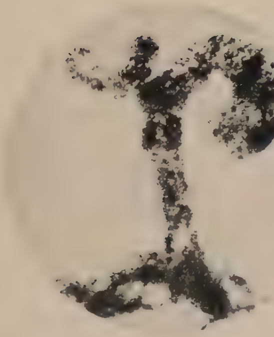

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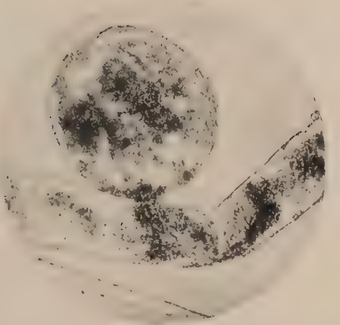

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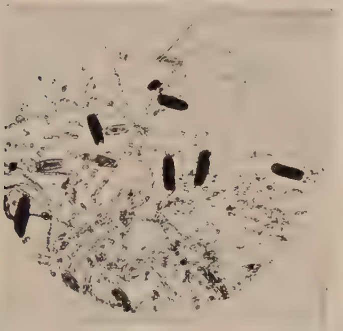

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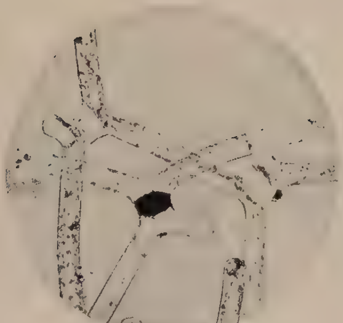

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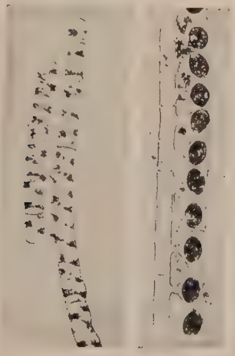

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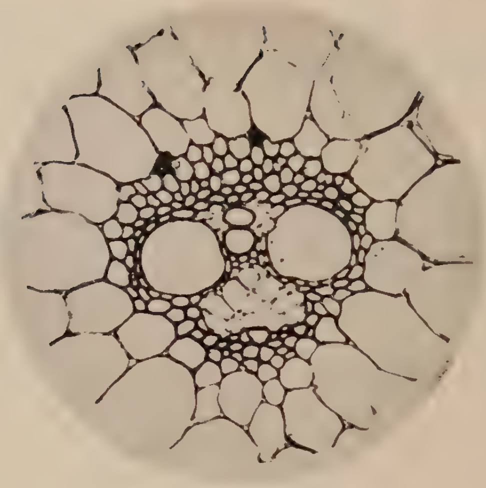

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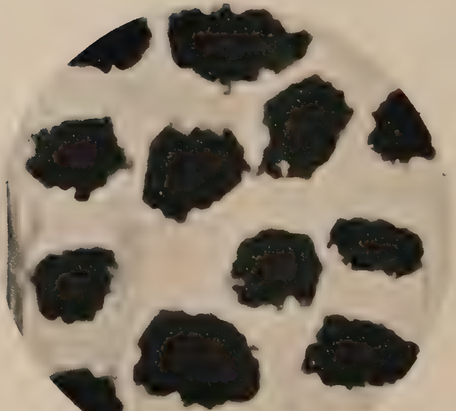

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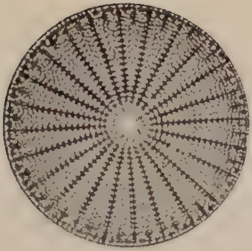
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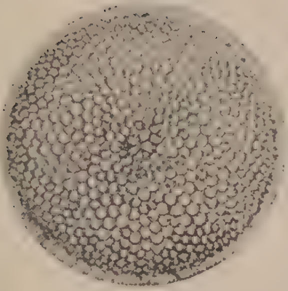
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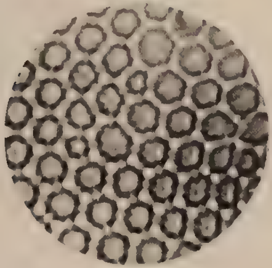
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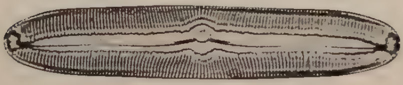
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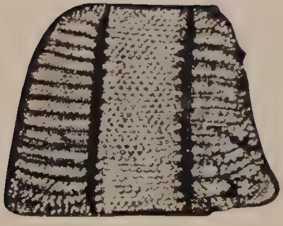
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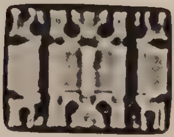
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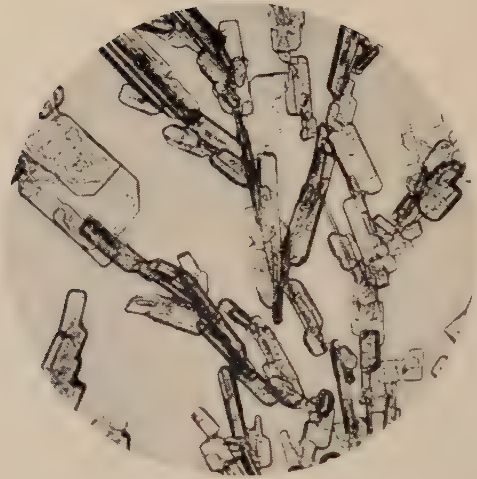

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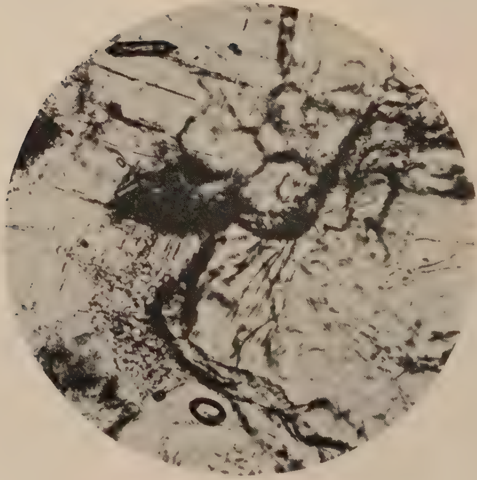

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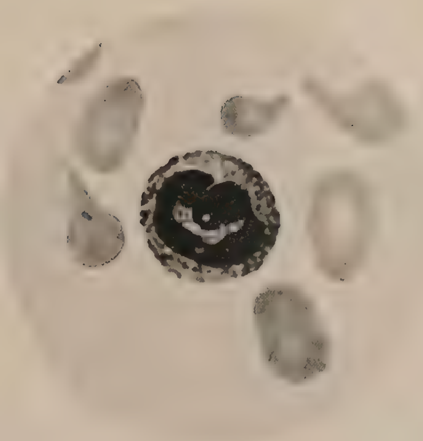

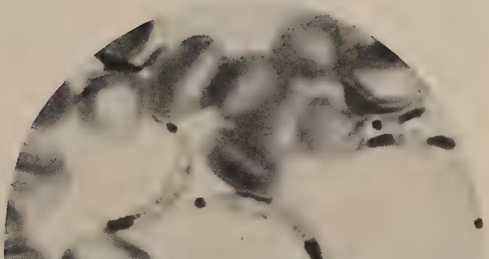

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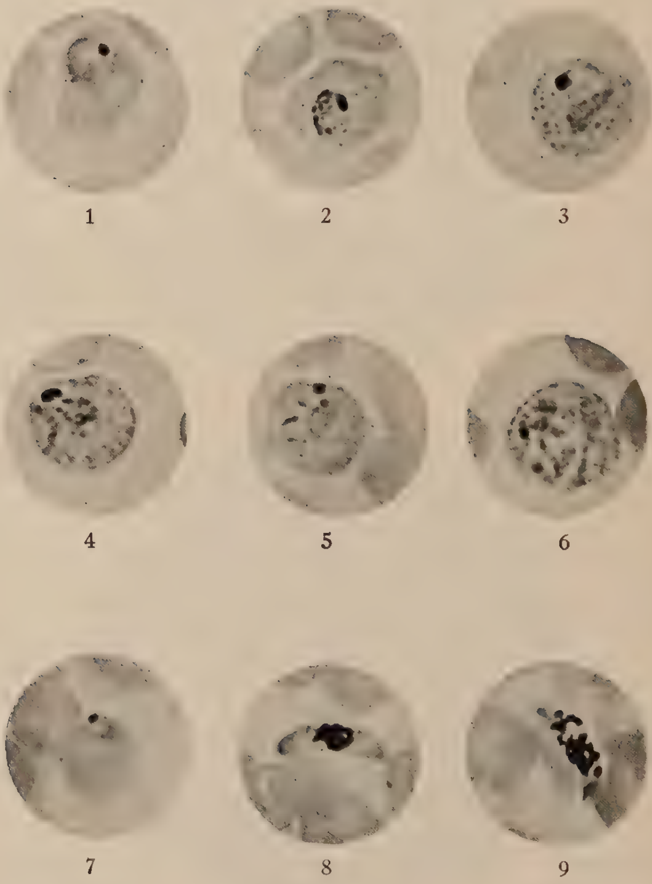

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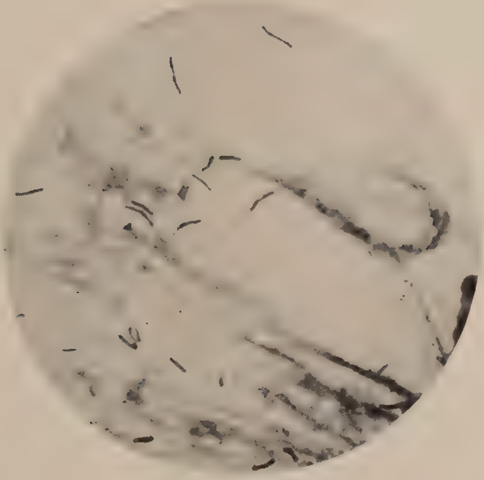

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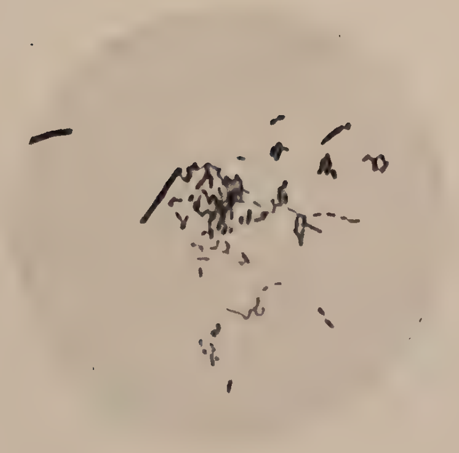

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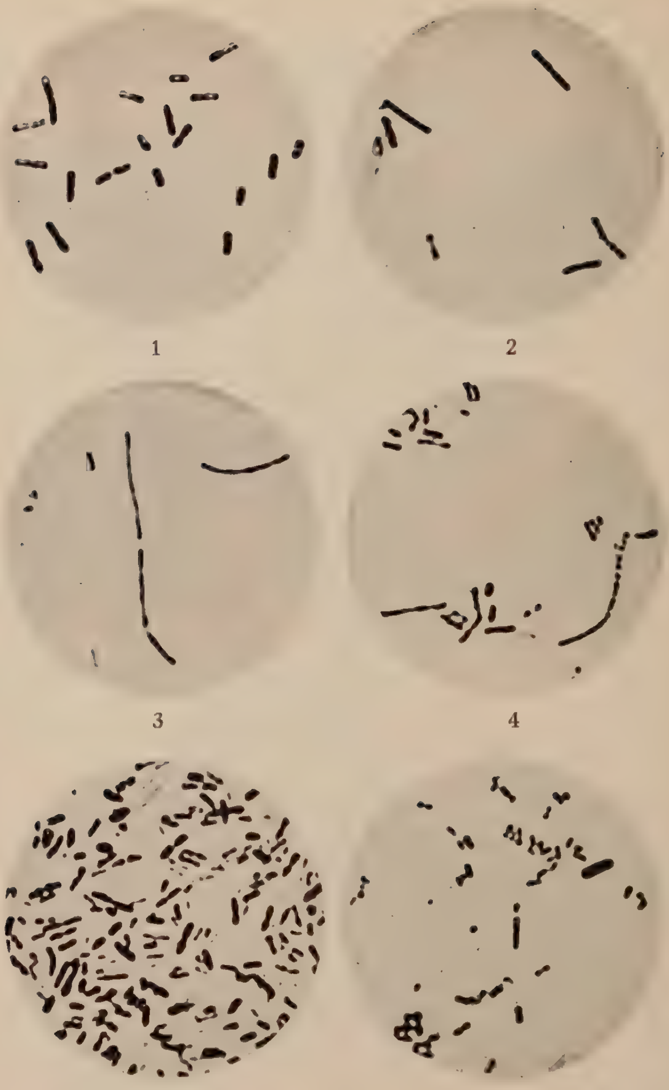

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